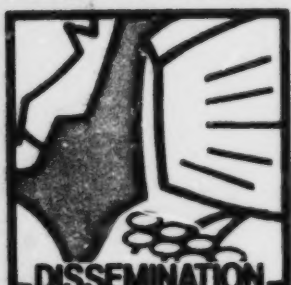


507.6-16

7 1978



ONCOLOGY OVERVIEW

SELECTED ABSTRACTS

COMPLETED

ON

ORIGINAL

STUDIES WITH VIRUS AND CELL MUTANTS IN TUMOR VIROLOGY

SEPTEMBER 19, 1978

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service • National Institutes of Health •
National Cancer Institute

A SERVICE OF THE
INTERNATIONAL CANCER RESEARCH DATA BANK (ICRDB)

Oncology Overviews are a service of the International Cancer Research Data Bank (ICRDB) program of the National Cancer Institute. Each *Oncology Overview* represents a survey of the literature associated with a selected area of cancer research. It contains abstracts of articles which have been selected and organized by researchers associated with the field. The purpose of this *Oncology Overview* is to facilitate and promote the exchange of information between cancer scientists by keeping them aware of literature related to their research being published by other laboratories throughout the world.

The material published in *Oncology Overviews* is obtained from Cancerline, an online cancer information system created by the ICRDB program in cooperation with the National Library of Medicine. Abstracts from the Cancerline data base, which is updated monthly with new articles abstracted from more than 1500 journals, can be retrieved via online computer terminals at more than 500 locations in the U.S. and other countries. Any organization linked to the Medlars Medline system at the National Library of Medicine has access to the Cancerline data base.

This *Oncology Overview* is sent automatically to researchers in the subject area who are identified on the basis of current research project descriptions submitted to an ICRDB-supported Current Cancer Research Project Analysis Center (CCRESPAC). We encourage all cancer researchers to provide a complete and current project description to CCRESPAC. If you have not recently supplied such a description to an agency providing input to this program (ie, NIH, American Cancer Society, Cancer Center Directors, etc.), you may obtain forms and additional information about data input from the ICRDB program office address listed below.

Oncology Overviews may also be obtained by any requestor at modest cost from the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, Virginia 22161, USA.

This *Oncology Overview* is prepared for the ICRDB program by the Cancer Information Dissemination and Analysis Center (CIDAC) for Cancer Virology, Immunology, and Biology (VIB). Further *Overview* editions depend on user needs and satisfaction. If this *Oncology Overview* is of value to you, please send your comments to:

CIDAC-VIB
Franklin Institute Research Laboratories
Science Information Services Department
20th and Race Streets
Philadelphia, Pennsylvania 19103, USA

If you would like to receive other ICRDB publications and learn about other ICRDB information services, please contact:

ICRDB Program Office, National Cancer Institute
Blair Building, Room 114, 8300 Colesville Road
Silver Spring, Maryland 20910, USA

EACH RESEARCHER RECEIVING THIS PUBLICATION IS URGED TO
CIRCULATE THIS *OVERVIEW* TO COLLEAGUES, CO-WORKERS, AND OTHERS
WHO ARE ENGAGED IN PROJECTS RELATED TO THE AREA COVERED BY
THIS PUBLICATION.

TABLE OF CONTENTS

ABSTRACT
NUMBER

INTRODUCTION

EDITORIAL COMMENTARY

I. THE IDENTIFICATION OF TUMOR VIRUS GENE FUNCTIONS USING VIRUS MUTANTS

A. Isolation and General Characterization of Virus Mutants	1-32
B. Genetic Analysis	33-44
C. Gene Mapping and Genome Structure of Virus Mutants	45-60
D. Identification and Study of Viral Gene Products	61-81

II. VIRAL MUTATIONS AFFECTING TRANSFORMING ABILITY

A. Mutations of DNA Tumor Viruses Affecting Transforming Ability	82-94
B. Mutations of RNA Tumor Viruses Affecting Transforming Ability	95-103

III. VIRAL MUTATIONS SPECIFICALLY AFFECTING THE EXPRESSION OF THE TRANSFORMED PHENOTYPE

A. DNA Tumor Virus Mutations and the Expression of the Transformed Phenotype	104-114
B. RNA Tumor Virus Mutations and the Expression of the Transformed Phenotype	115-128

IV. HOST CELL MUTATIONS OR VARIATIONS AFFECTING THE INTERACTION WITH TUMOR VIRUSES

A. Reversion of Transformed Cell Properties	129-165
B. Conditional Expression of Transformed Cell Properties	166-175
C. Effect of the Host Mutations/Variations on the Expression of Viral Functions ...	176-184

V. STATE AND EXPRESSION OF THE VIRAL GENOME IN VIRUS TRANSFORMED CELLS

A. Virus Integration	185-191
B. Regulation of Induction of Loss of Viral Genomes	192-206
C. Expression of Viral Genes	207-214

VI. ANALYSIS OF TRANSFORMED CELL PROPERTIES USING VIRUS AND CELL MUTANTS

A. Surface Properties of Transformed Cells	215-250
B. Transport Properties of Transformed Cells	251-259
C. Cytoskeletal Structures in Transformed Cells	260-267
D. Cyclic Nucleotides in Transformed Cells	268-276
E. Growth Properties/Cell Cycle Characteristics of Transformed Cells	277-285
F. Tumor Antigens of Transformed Cell Properties	286-291
G. Other Alterations of Transformed Cell Properties	292-302

VII. REVIEWS AND GENERAL ARTICLES

303-330

SUBJECT INDEX

AUTHOR INDEX

INTRODUCTION

This ONCOLOGY OVERVIEW on studies with viral and cellular mutants in tumor virology covers the publications years from 1970 to the recent entries in the CANCERLINE data base. In some cases, selected information from the 1960's is included for historical value. The strategy used for the online data base search is available on request from the CIDAC office and can be entered at any MEDLARS/MEDLINE terminal system in order to obtain continuing updates in this area.

The scope of this overview covers the subject of mechanism of neoplastic transformation by viruses with an emphasis on studies conducted with the help of genetic tools, i.e., viral or cellular mutants. Investigations examining the altered properties of transformed cells, the characterization of viral and cellular mutants, and the role of viral and cellular functions in the phenomenon of transformation will be included.

This overview was prepared by Claudio Basilico (Editor) at the Department of Pathology, New York University School of Medicine and Barbara Franklin at the CIDAC for Virology, Immunology and Biology, The Franklin Institute Research Laboratories.

BLANK PAGE

EDITORIAL COMMENTARY

While the demonstration that certain viruses can cause tumors in animals dates back to the beginning of the century, only in the past fifteen years has wide attention been paid to the study of carcinogenesis with DNA and RNA tumor viruses. During this period the field has undergone rapid expansion in many different directions. First, tumor viruses have been studied as a model system for carcinogenesis. Because of the relative ease with which the primary interaction of these viruses and their hosts (particularly *in vitro*) can be studied, this has permitted: (1) the dissection of the viral functions responsible for cell transformation, (2) the identification of the viral gene-products involved, and (3) the possible identification of the mode of action of these viral gene-products. A second direction for tumor virus study has been investigation of the possible viral etiology of human cancers. A third major area in the study of tumor viruses has focused on the regulation of normal cell growth. Since such regulation is perturbed in cells infected or transformed by tumor viruses, such studies could provide an insight into the mechanisms of normal growth control.

The successes and occasional disappointments encountered in this field of research are familiar to many people. Though tumor virus research has been extremely useful in identifying basic mechanisms in eukaryotic molecular biology and in some cases has contributed to the development of important basic research techniques, the mechanism by which viruses convert a normal cell into a cancer cell remains elusive. Yet, considerable progress toward the solution of this formidable problem has been made. An understanding may soon surface as to how and what gene or gene-products are responsible for the tumorigenic potential of viruses.

To say that genetic analysis and genetic tools (i.e. mutants) have played a crucial role in the development of this field of research is probably an understatement. Genetic analysis of tumor viruses and their host cells has been vital in identifying the genes and steps involved in transformation and the variety of controls that can influence the expression of the neoplastic phenotype. Without such a combined genetic and biochemical approach, progress in this field would have been considerably slower. In some cases, investigations have unearthed new and unsuspected phenomena underlying the complexities of the entire system; in other cases, this research has provided a solid foundation for now widely-accepted theories.

Historically, the thrust of the genetic approach to tumor virology has been the identification of the viral gene or gene product(s) directly responsible for transformation. In this respect, the small DNA tumor viruses, such as polyoma or SV40, have been widely studied because the genome of these viruses is quite small (about 5000 base pairs). Such a limited amount of genetic information is likely to code for no more than 5-8 average size proteins, and by isolating mutant strains it appears possible to define all the genes in these viruses, and to identify the polypeptide each gene specifies, including the one(s) causing transformation.

Genetic analysis of these small DNA viruses has led to the identification of probably all their genes and of the proteins they specify, but the relative involvement of each gene in transformation is still not completely clear. One gene (called gene A) has been conclusively associated with the establishment of transformation. This gene codes for a DNA binding protein of high molecular weight, which is found in the nucleus of infected or transformed cells, and is generally known as T-antigen. However, its importance in maintaining the transformed phenotype is still a subject of controversy, and it is possible that at least another viral protein is involved in the process of cell transformation.

In the case of RNA tumor viruses, the identification of a gene directly responsible for cell transformation has been easier, possibly because in these viruses the reproductive and the cell transforming capacity can be separated. Thus, deletions which abolish the transforming but not the reproductive ability of RNA sarcoma viruses were readily found. Most important, viral temperature sensitive mutations were isolated which make the phenotype of the transformed cells temperature dependent. Cells transformed by these mutants behave like transformed cells at low temperature and like normal cells at high temperature. The isolation of these mutants was an important event because it established beyond question that transformation by RNA tumor viruses requires the continuous presence and expression of viral genes. In

Editorial Comment

this case however, while the gene responsible for neoplastic transformation is known, the protein it specifies still remains elusive.

While these studies have shown clearly that viral gene-products are responsible for the initiation of cell transformation, the complexity of the system has been underlined by studies with cellular mutants. It has become clear that a variety of host mutations or variations can suppress the expression of the transformed phenotype in cells containing an intact and functioning viral genome. Thus, the host cell also exerts control on the expression of the transformed phenotype. Finally, viral and cellular mutants have been widely used to probe other characteristics of viral transformation, such as the control of the integrated state of the viral DNA in transformed cells, the regulation of gene expression, and the study of the altered properties characteristic of transformed cells.

This OVERVIEW is an attempt to assemble and usefully organize abstracts of the most significant work published in the past decade concerning the genetic approach toward viral transformation. The papers abstracted cover areas ranging from the characterization of viral and cellular mutants, and the study of the involvement of one or more viral and cellular functions in the phenomenon of transformation, to the investigation of the altered properties of transformed cells conducted with the use of specific cellular and viral mutants.

We hope this OVERVIEW will be a useful reference, not only for scientists working in this field, but also for scientists and teachers whose interests lie in related areas.

I. THE IDENTIFICATION OF TUMOR VIRUS GENE FUNCTIONS USING VIRUS MUTANTS

A. Isolation and General Characterization of Virus Mutants

1. THE DEFECTIVENESS OF ROUS SARCOMA VIRUS (MEETING ABSTRACT).

Hanafusa H, Hanafusa T, Rubin H
Virus Lab., U. Cal., Berkeley
Proc Natl Acad Sci USA; 49(4):572-580 1963

Foci of chick embryo cells, transformed by high titer Rous sarcoma virus (RSV) made free from Rous-associated virus (RAV), produced no RSV on repeated transfer. Such non-producing (NP) cells were morphologically Rous sarcoma cells, but sonic vibration and X-irradiation could not cause the release of virus. Infection of NP cells with RAV, however, (5×10^7 infectious units) resulted in abundant release of RSV within one day. RSV was never produced in the absence of RAV and was always initiated (in NP cells) on superinfection with RAV. Further experiment showed the absence of RSV production in the early stages of solitary infection. An inoculum of 100 FFU of RSV produced 6160 foci in 5 days, but only 238 when irradiated (probably from contamination with RAV). A similar inoculum plus 10^7 infectious units of RAV produced 7400 foci in 4 days, 7300 when irradiated. The ability of RAV to initiate RSV production was duplicated by all the viruses of the avian leukosis complex tested, including a visceral lymphomatosis virus (RIF), myeloblastosis virus, and an RAV-like agent isolated from standard RSV. It is concluded that the high titer strain (and probably the standard strain as well) of RSV virus is a defective virus, incapable of producing infective progeny in a solitary infection, and that RAV plays the role of a helper virus when multiplying in the same cell. Both capacities are perpetuated, in a cell line, by hereditary transmission. A possible relationship is suggested between the defectiveness of RSV and its carcinogenic properties. The failure to produce mature virus could result in unrestrained production of the early virus products, thus impairing the regulatory functions of the cell.

2. ANALYSIS OF THE DEFECTIVENESS OF ROUS SARCOMA VIRUS. III. DETERMINING INFLUENCE OF A NEW HELPER VIRUS ON THE HOST RANGE AND SUSCEPTIBILITY TO INTERFERENCE OF RSV.

Hanafusa H
Dept. Exp. Med., Coll. France, Paris
Virology; 25(2):248-255 1965

A second helper virus was isolated from Rous sarcoma virus (RSV) no. 8 stock and designated as RAV-2 (the original strain of RAV reported in CRA 2(2):#282, 1964 is now designated as RAV-1). RAV-2 and RAV-1 are antigenically unrelated. RAV-2 grows in cells of phenotype K but not in cells of phenotype K/2, whereas RAV-1 grows equally well in both cell types. RSV activated by RAV-2 is not susceptible to interference (with infection by RSV) imposed by RAV-1. It is concluded that the host range properties of RSV, its susceptibility to interference, and its antigenicity are determined by the helper virus and are therefore probably controlled by the virus coat. (For earlier studies, see CRA 2(1):#85; and *ibid*, (2):#282, 1964)

3. TEMPERATURE SENSITIVE MUTANTS OF AN AVIAN SARCOMA VIRUS.

Toyoshima K, Vogt PK
U. Washington Sch. Med., Seattle
Virology; 39(4):930-931 1969

Chick embryo fibroblasts were inoculated with 106 FFU of B77 virus, and on day 5, the transformed virus-producing cells were exposed to 5-azacytidine (25 microg/ml; at 36 C x 24 hours). Mutagenized virus was then plated onto type C/O chick embryo fibroblasts at the permissive temperature (36 C). Most of the virus clones produced had reduced focus-forming efficiency and yield at the non-permissive temperature (40.5 C), but 2 were strongly suppressed. No foci of transformed cells were observed in cultures incubated with the temperature-sensitive mutants at the non-permissive temperature, or shifted to non-permissive temperatures after 20 hours. Foci induced by the virus disappeared after shifting to non-permissive temperatures as late as 20 hours after infection. The temperature-sensitive mutants also could not replicate at the non-permissive temperature.

4. THE REPRODUCTIVE AND CELL-TRANSFORMING CAPACITIES OF AVIAN SARCOMA VIRUS B77: INACTIVATION WITH UV LIGHT.

Toyoshima K, Friis RR, Vogt PK
Res. Inst. Microbial Dis., U. Osaka, Japan
Virology; 42(1):163-170 1970

A strain of avian sarcoma virus (Bratislava 77) was isolated from a virus-producing rat cell line subjected to UV irradiation to investigate the ability of UV-irradiated viruses to reproduce and to transform cells. Following UV-irradiation, 2 classes of radiation-damaged survivor virus particles were detected. The ability to release free virus from foci formed by UV-irradiated survivor was also impaired even in the presence of an avian leukosis virus helper. During 5-8 transfers of foci from these survivors a gradual disappearance of transformed cells from the culture was observed, indicating that there may have been a reversion to normal cell morphology due to death of transformed cells. The second class of particles could not transform cells, but produced pseudotypes with a strain of Rous sarcoma virus, interfered with avian sarcoma viruses of subgroup C, and was antigenically identical to the original strain of irradiated virus.

5. RADIO-INDUCED MUTANTS OF THE SCHMIDT-RUPPIN STRAIN OF ROUS SARCOMA VIRUS.

Golde A
Radium Biol. Inst., Orsay, France
Virology; 40(4):1022-1029 1970

In a study of pock cultures, the virus-producing capacity of clonal and subclonal Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV) irradiated with ^{60}Co showed a 0-25% increase of nonproducing (NP) transformed cells as irradiation dosage increased. Of those focus cultures that remained virus-producers, the smaller virus yield occurred at higher irradiation dose. NP cells, obtained by inoc of irradiated viral suspensions either on the chorioallantoic membrane (CAM) or on chicken cells in vitro, induced graft tumors on the egg CAM. A group of mutant virions, which were unable to transform C/O chick embryo cells, but were able to replicate at high titer and induce high group specific antigen levels, were obtained. This mutant confers to the cells a high resistance against RSV challenge (subgroups B and D of avian oncogenic

viruses), and a slight resistance against subgroup A RSV challenge. The hypothesis of the independence of oncogenic and virus-producing capacities is reaffirmed.

6. CONDITIONAL LETHAL MUTANTS OF AVIAN SARCOMA VIRUSES: I. PHYSIOLOGY OF TS 75 AND TS 149.

Friis RR, Toyoshima K, Vogt PK
Virology; 43(2):375-380 1971

Physiological activity of 2 temperature-sensitive mutants of avian virus was analyzed by means of complement-fixation and uridine labeling of viral RNA. The half-life for the viruses tested at 41 degrees was 60 min, indicating inactivation kinetics indistinguishable from wild type viruses. Cells transformed by the mutants were only 10% as effective in establishing tumors compared to the tumor-producing ability of the wild type-transformed cells. Rates of heat inactivation, envelope antigenicity and host range were identical to the wild type. Reversion frequencies to wild types were 3×10^{-6} for ts 75 and 4×10^{-6} for ts 149. Uridine labeling indicated that ts 75 infected cells synthesized RNA at 41 degrees in substantial amounts 96 hr after infection. Whether DNA synthesis needed for virus production and for transformation can occur at 41 degrees remains to be investigated.

7. A TEMPERATURE-SENSITIVE MUTANT OF ROUS SARCOMA VIRUS THAT IS DEFECTIVE FOR REPLICATION.

Friis RR, Hunter E
U. Southern California Sch. Med., Los Angeles
Virology; 53(2):479-483 1973

The isolation and properties of ts 672, a temperature sensitive mutant of the Prague strain of Rous sarcoma virus which fails to replicate infectious progeny at 41 C, are described. Chick embryo cells infected with ts 672 are transformed, as may be seen by focus formation and increased rate of 2-deoxy-glucose transport. Ts 672 is defective for some function necessary to the maturation of infectious progeny. This function is complemented by nontransforming viruses such as the Rous associated viruses. Ts 672-infected cells produce viral type-specific antigen which is recognized by antiserum specific to subgroup A, and which is effective in establishing interference against other viruses of subgroup A. The infected cells also produce physical particles that incorporate 3H-uridine and cosediment with avian tumor viruses on sucrose density gradients in large numbers.

8. ISOLATION OF DEFECTIVE MUTANT OF AVIAN SARCOMA VIRUS (MEETING ABSTRACT).

Kawai S, Hanafusa H
Public Hlth. Res. Inst. City of New York, N.Y.
Proc Natl Acad Sci USA; 70(12):3493-3497 1973

A colony of transformed cells was isolated from chick-embryo cells infected with a stock of nondefective Schmidt-Ruppin strain of Rous sarcoma virus. The virus recovered from this colony was a stable defective mutant very similar to the Bryan strain of Rous sarcoma virus in the following characteristics: noninfectiousness of virus particles released from transformed cells that lack helper factor; formation of infectious pseudotypes by coinfection with avian leukosis virus or by interaction with endogenous-helper factor in chicken cells; ability of the noninfectious form of virus to transform chick-embryo cells in the presence of ultraviolet light-inactivated Sendai virus; absence of glycoprotein in the noninfectious form; failure to produce nondefective virus by recombination with avian leukosis virus and segregation of polymerase-negative virus. The morphology of the transformed cells is characteristic of those infected by the Schmidt-Ruppin strain. The demonstration of segregation of such a defective virus from nondefective sarcoma virus and

failure to detect revertants of this mutant suggest that the deletion of some genes may be involved in this mutation.

9. ISOLATION OF TEMPERATURE-SENSITIVE MUTANTS OF MURINE LEUKEMIA VIRUS.

Stephenson JR, Reynolds RK, Aaronson SA
Natl. Cancer Inst., Bethesda, Md.
Virology; 48(3):749-756 1972

Kirsten murine leukemia viruses (KiMuLV) growing in NIH/3T3 mouse cells were mutagenized by treatment with 5-bromodeoxyuridine (BrdU) or N-methyl-N'-nitro-N-nitrosoguanidine (NTG). A total of 407 NTG-mutagenized KiMuLV-infected clones, and 410 BrdU-treated infected clones, were screened by a microtiter technique for the presence of temperature-sensitive (ts) mutants. Twenty-eight of the NTG-treated clones and 13 of the BrdU-treated clones harbored potential ts mutants, as evidenced by inability to induce syncytia on rat tumor cells (XC) at 31 C (a nonpermissive temperature). Of the 41 potential ts mutant clones, nine (two from BrdU- and seven from NTG-treated clones) were subsequently shown to be ts mutants. Each of the nine ts mutants was investigated to quantitate rates of spontaneous reversion at a permissive temperature. The reversion rates for each of the ts mutants were very low.

10. AN AVIAN SARCOMA VIRUS MUTANT THAT IS TEMPERATURE SENSITIVE FOR VIRION ASSEMBLY.

Hunter E, Hayman MJ, Rongey RW, Vogt PK
Univ. Southern California Sch. Medicine, 2025 Zonal Ave.,
Los Angeles, CA 90033
Virology; 69(1):35-49 1976

The temperature-sensitive defect in replication of LA334, a double mutant of Rous sarcoma virus, has been characterized both biologically and biochemically. This mutant is complemented for replication at the nonpermissive temperature by both leukosis virus and by RSV(-). Both experiments indicate that LA334 synthesizes functional glycoproteins at the restrictive temperature. In the following experiments, the recombinant virus LA3342 was used; this mutant transforms cells at both 35 C and 41 C. Cells were infected at 35 C, then shifted to 41 C when confluent infected. Interference experiments showed that LA3342 induced resistance at 41 C against superinfection with viruses of subgroup C. Noninfectious virus particles were synthesized at 41 C and had a higher density in sucrose gradients (1.175 g/ml) compared to those produced at 35 C (1.15 g/ml). These noninfectious virions contained four novel polypeptides; at least three appeared to be viral in origin. The rate of cleavage of the polypeptide precursor to nonglycosylated structural polypeptides was reduced significantly at the restrictive temperature. Addition of cycloheximide to infected cultures did not prevent the rapid production of infectious virus, seen on shifting cells from the restrictive to the permissive temperature. This finding suggests that proteins synthesized at 41 C can be processed correctly at 35 C. Large budding structures lacking the characteristic morphology of C-type particles were visible in electron micrographs of cells infected with the mutant at the nonpermissive temperature. The mutation affecting replication in LA3342 appears to be in the gene coding for one of the nonglycosylated structural proteins.

11. HEREDITARY MODIFICATIONS OF AVIAN SARCOMA VIRUSES BY QUAIL CELL PASSAGES.

Toyoshima K, Nomaguchi H, Owada M, Ihara S, Yoshida M
Res. Inst. Microbial Diseases, Osaka Univ., Yamada-kami,
Suita, Osaka, Japan
Bibl Haematol; (43):453-455 1976

Attempts were made to adapt mutants of avian sarcoma viruses to quail embryo fibroblasts. The viruses used were

wild-type and temperature-sensitive (ts) LA334 of B77 C, and wild-type and ts OS 538 of SR-RSV-A and PR-RSV-A. After repeated cloning of these viruses on quail embryo fibroblasts, their transforming character was modified. The recombinants induced fusiform or round-plus-fusiform type transformation not only on quail embryo fibroblasts but also on chick embryo fibroblasts. Two LA334-derived recombinants induced fusiform type transformation on chick and quail embryo fibroblasts even at the nonpermissive temperature, although the original LA334 did not produce foci under the same conditions. The modified character was analyzed further by studying the effect of the recombinants on the adenylate cyclase activity of chick embryo fibroblasts. A wild-type B77 recombinant induced changes of activity at both the permissive and nonpermissive temperatures. The LA334 recombinants induced similar changes at the permissive temperature. At the nonpermissive temperature, however, the Km value of the cyclase was intermediate between those of uninfected and transformed cells. This change seems to be the result of quail passage, because SR-RSV-A reduced the adenylate cyclase activity of chick embryo fibroblasts but did not affect the Km value of the enzyme for ATP. One clone of this virus, however, acquired the ability to change the Km value for ATP after passage through quail embryo fibroblasts.

12. STUDIES ON THE LYTIC INTERACTION AND CELL TRANSFORMATION WITH A LARGE- AND A SMALL-PLAQUE MUTANT POLYOMA VIRUS.

Medina D, Sachs L

Weizmann Inst. Sci., Rehovoth, Israel

Virology; 19(2):127-139 1963

The comparative lytic efficiency of small-plaque (SP2) and large-plaque (BP5) polyoma virus mutants was determined from growth curves of 1-day-old secondary cultures containing 1×10^6 cells, inoculated with 0.1 ml of each stock virus at a virus: cell ratio of 10 plaque forming units (PFU)/cell. (Virus titration was by plaque assay on C57BL/6 secondary mouse embryo monolayers) The total virus (TV) content of both mutants, on mouse embryo monolayers, increased between 24 and 36 hr, but at 72 hr value for BP5 was 1.7×10^8 PFU; for SP2, 1.4×10^9 PFU. The SP2 mutant also showed greater TV increase on hamster embryo cells. SP2 was also more efficient in virus adsorption during the 3 hr infection period. The percentage of SP2-infected mouse embryo cells was 3.8-7 times higher than after infection with BP5: in hamster cells, yield was 4.2 to 20 times higher. Average virus yield per virus-yielding cell was equal for the 2 mutants ($2-4 \times 10^3$ PFU). The small plaque size of SP2 showed no change with addition to the agar nutrient overlay of either DEAE-dextran or protamine. In the study of cell transformation, the percentage of transformed clones (PTC) was determined by cloning cultures of hamster cells after exposure to virus at 20 PFU/cell. PTC for BP5 was 0.1%-0.4%; for SP2, 0.8%-2.0%. Comparable results were obtained after multiple exposures to the virus, and after a single exposure to a more concentrated inoculum (1000 PFU/cell). However, PTC was increased to 10.0% for SP2 and 3.0% for BP5 in cultures maintained at 24 C for 24 hr before or after exposure to the virus. These results indicate that the rate of polyoma virus-induced cell transformation can be altered by choice of viral mutant and changes of culture conditions.

13. TEMPERATURE-SENSITIVE MUTANTS OF POLYOMA VIRUS.

Di Mayorca G, Callender J, Marin G, Giordano R

Int. Lab. Genet. Biophys., Naples, Italy

Virology; 38(1):126-133 1969

Of 150 temperature-sensitive polyoma virus mutants isolated and purified, 95% were stable and maintained their

characteristics through several passages; for 33% of the mutants, however, the yield at the permissive temperature (30 C) was only 101-102 times greater than that at the nonpermissive temperature (38.5 C). Over 50% of the mutants showed no complementation at all, while the rest belonged to 4 complementation groups (genes). The first group corresponded to a gene involved in viral DNA synthesis (mutants of this group showed temperature-sensitive transforming ability); the second and third groups corresponded to genes coding for viral proteins. The function of the fourth group (only 1 mutant belonging to this group is listed) has not been determined.

14. COMPLEMENTATION AND TRANSFORMATION BY TEMPERATURE-SENSITIVE MUTANTS OF POLYOMA VIRUS.

Eckhart W

Salk Inst. Biol. Studies, San Diego, Calif.

Virology; 38(1):120-125 1969

Six temperature-sensitive mutants of polyoma virus (PV) were isolated and grouped into 3 classes, on the basis of complementation and transformation at the non-permissive temperature (38.5 C) in secondary mouse embryo cell monolayers. Class I mutants (2 were isolated) synthesized infectious viral DNA but not mature PV particles, and showed normal transformation (in BHK cells). The 3 Class II mutants were defective in both infectious viral DNA synthesis and in transforming ability. The single mutant of Class III transformed normally, but was defective in the synthesis of infectious viral DNA.

15. CHARACTERIZATION OF A TEMPERATURE-SENSITIVE MUTANT OF POLYOMA VIRUS.

Fried M

Imperial Cancer Res. Fund, London

Virology; 40(3):605-617 1970

Incubation of mouse embryo cells, infected with a temperature-sensitive mutant of polyoma virus (TS-a), at the nonpermissive temperature (38.5 C), inhibited production of progeny virus. Production of progeny virus increased almost to the level of that induced by the parental strain as the input multiplicities increased from 0.1-100 PFU/cell. There was no significant difference in the heat denaturation of the capsid protein or extracted DNA compared to the parental virus, nor was the temperature-sensitive step due to alterations in adsorption, release or uncoating; however, production of infectious DNA in TS-a-infected cells was decreased. Shifting to non-permissive temperatures at various times during the growth cycle in order to determine when the temperature sensitive step occurred showed that the virus yield started to become resistant to the shift 15-23 hours after infection, and was completely resistant after 87 hours when only 40% of the final virus yield was completed.

16. TRANSFORMATION OF MOUSE 3T3 CELLS BY T ANTIGEN-FORMING DEFECTIVE SV40 VIRIONS (T PARTICLES).

Uchida S, Watanabe S

Nat. Inst. Health, Shinagawa-ku, Tokyo

Virology; 39(4):721-728 1969

Mouse 3T3 cells infected with defective deletion mutants of SV40 or plaque-formers had a transformation frequency directly proportional to the virus dose up to 3×10^8 T antigen-forming units. Cells from colonies showed loss of contact inhibition, were positive for T antigen, were virus-free, but

were negative for V antigen and only had weak anti-T fluorescence. These cells were fused with African green monkey kidney cells and the resulting heterokaryons produced V antigen only when derived from the plaque-formers. The V antigen-producing heterokaryons demonstrated an increase in anti-T fluorescence, possibly due to viral replication. It is suggested that the strongly fluorescent T-antigen in the nucleus of the heterokaryons from the defective virus was due to replication of the defective T particle genome. Nuclear alterations in the heterokaryons are described.

17. TEMPERATURE-SENSITIVE SIMIAN VIRUS 40 MUTANT DEFECTIVE IN A LATE FUNCTION.

Kit S, Tokuno S, Nakajima K, Trkula D, Dubbs DR
Baylor Coll. Med. Houston, Tex.
J Virol; 6(3):286-294 1970

A cell-line of monkey kidney cells was infected with a strain of SV40 (infection multiplicity of 5 plaque-forming units/cell in a 0.2 ml volume) and then treated with the mutagen nitrosoguanidine in doses of 100 microg/ml culture for the purpose of isolating a SV40 mutant which replicated at differing rates in differing temperature conditions. Replication of virus, designated tsTNG-1, at the nonpermissive temperature (38.7 C) was 3,000-fold less than at the permissive temperature (33.5 C). Plaque formation by the mutant strain's DNA on kidney cell culture monolayers occurred normally at 33.5 C but was grossly inhibited at 38.7 C. The time at which virus replication was blocked at 38.7 C was determined by temperature-shift experiments. In shift-up experiments, cultures infected for various times at 33.5 C were shifted to 38.7 C. In shift-down experiments, cultures infected for various times at 38.7 degrees C were shifted to 33.5 C. All cultures were harvested at 96 hr postinfection. No virus growth occurred when the shift-up occurred before 40 hr postinfection. Maximum virus yields were obtained at 96 hr postinfection when the shift-down occurred at 66 hr, but only 15% of the maximum yield was obtained when the shift-down occurred at 76 postinfection. These results indicate that tsTNG-1 contains a conditional lethal mutation in a late viral gene function. The mutant synthesized tumor antigen, viral capsid antigens, and viral DNA, and induced thymidine kinase activity at either 33.5 or 38.7 C. The properties of the viral DNA synthesized in mutant-infected kidney cell culture cells at 33.5 or 38.7 C were very similar to those of SV40 DNA made in parental virus-infected cells, as determined by nitrocellulose column chromatography, cesium-chloride-ethidium bromide equilibrium centrifugation, and by velocity centrifugation in neutral sucrose gradients. Mutant tsTNG-1 enhanced cellular DNA synthesis in primary cultures of mouse kidney cells at 33.5 and 38.7 C and also transformed mouse kidney cultures at 36.5 C. After fusion with susceptible monkey kidney cell cultures and incubation at 38.7 C, the mutant could not be recovered from a clonal line of transformed cells; however, after incubation at 33.5 C, recovery was possible.

18. ISOLATION AND CHARACTERIZATION OF TEMPERATURE-SENSITIVE MUTANTS OF SIMIAN VIRUS 40.

Kimura G, Dulbecco R
Tottori Univ. Sch. Medicine, Yonago, Japan 683
Virology; 49(2):394-403 1972

Six temperature-sensitive (ts) mutants of two wild-type strains of Simian Virus 40 (SV40) were isolated using either hydroxylamine or nitrosoguanidine as mutagens; the ts mutants were able to produce plaques at 33 C but not at 40 C. Mutant plaques produced at 33 C were similar in size and shape to wild-type plaques produced at 33 C or 40 C. Anti-SV40 rabbit serum inhibited the plaque-forming ability of all six mutants as well as of the wild-type virus at a 1:500 dilution. A single cycle

of virus development at 33 C or at 40 C produced equal yields of wild-type viruses, but the yield of mutants was 26-13,000-fold lower at 40 C than at 33 C. Infection with mutant viral DNA alone also produced lower yields at 40 C than at 33 C; this finding indicates that the lower yield at 40 C cannot result from a defect in viral absorption, penetration or uncoating at the non-permissive temperature, but must result from a defect in the intracellular growth cycle of the virus. Both mutant and wild-type viruses synthesized about the same amount of infectious viral DNA at both permissive and non-permissive temperatures. But the infectivity of the six mutants was destroyed much more rapidly than that of the corresponding wild types, suggesting that the mutants have defects in structural genes coding for virion components; these components were tentatively assigned to two loci on the basis of complementation assays. The ability of the mutants to transform rat 341 cells was not altered in comparison with the wild-type strains. Given the size of the SV40 genome, it can code for five to eight small proteins; this study reports on two of them. (22 Refs)

19. GENETIC ANALYSIS OF SIMIAN VIRUS 40. III. CHARACTERIZATION OF A TEMPERATURE-SENSITIVE MUTANT BLOCKED AT AN EARLY STAGE OF PRODUCTIVE INFECTION IN MONKEY CELLS.

Robb JA, Martin RG
Natl. Inst. Arthritis Metab. Dis., Bethesda, Md.
J Virol; 9(6):956-968 1972

TC7 cells, a cloned subline of CV-1 monkey cells, were inoculated with the ts 101 (101) temperature-sensitive mutant of SV40. 101 virions adsorbed as efficiently as nonmutant wild-type SV40 to inoculated cells at restrictive (40-41 C) as well as at permissive (30-33 C) temperatures. 101 virions also penetrated cells normally at restrictive temperatures (i.e. they lost sensitivity to neutralizing antibody after adsorption to cells). At restrictive temperatures, the 101 virions were unable to induce cellular DNA synthesis or synthesis of viral DNA and the viral antigen. SV40-specific tumor antigen synthesis and SV40 U-antigen synthesis were also inhibited in 101 cells at the restrictive temperature. First cycle tumor- and viral-antigen synthesis after infection of cells with 101 DNA were normal at the restrictive temperature, but the resulting progeny virus were as temperature sensitive as their 101 parents. 101 virions did not complement or inhibit other temperature-sensitive SV40 mutants or wild-type virions. The affected protein in 101 virions may be a regulatory structural protein, perhaps a core protein, that interacts with the DNA of the virions.

20. MUTANT OF POLYOMA VIRUS WITH IMPAIRED ADSORPTION TO BHK CELLS.

Basilico C, DiMayorca G
New York U. Sch. Med., N. Y.
J Virol; 13(4):931-934 1974

A mutant of polyoma virus (PY), PY 235, was isolated as a temperature-sensitive (TS) mutant from a stock of large-plaque (LP) PY virus. Although PY 235 is somewhat TS in plaque formation, it shows no temperature sensitivity in terms of growth. When PY 235 was tested for transforming ability using hamster BHK-21 and rat ReCL3 cells, it did not cause any detectable transformation at any temperature. Thus, PY 235 appears to have a transformation defect. The mutant virus was unable to cause any significant hemagglutination (HA) in guinea pig RBC; this was due to a lack of adsorption of the virus to RBC. The adsorption of 3H-labeled wild type (WT) and 235 PY virus to 3T3 and BHK cells was tested. The adsorption of PY 235 to mouse cells was slow, reaching only about 40% of the WT values. Adsorption to BHK cells was much more inhibited, reaching no more than 5% of the WT values. The lack of transformation observed with PY 235 was probably due solely to a lack of adsorption to BHK cells. The

partial temperature sensitivity of PY 235 can also be explained in terms of impaired adsorption and reinfection of neighboring cells at 39 C.

21. PROPERTIES OF TEMPERATURE-SENSITIVE MUTANTS OF POLYOMA VIRUS.

Eckhart W

Tumor Viruses Cold Spring Harbor Symposia on Quantitative Biology; Cold Spring Harbor Laboratory (Cold Spring Harbor, NY) (39):37-40 1975

The properties of various temperature-sensitive mutants of polyoma virus were characterized by studying their ability to transform BHK, 3T3, and Swiss mouse embryo cells. Two classes of mutants were detected: (1) early mutants blocked in viral DNA synthesis at the restrictive temperature, and (2) late mutants that synthesize viral DNA, but not viral particles, at the restrictive temperature. The author discusses the available literature concerning the properties of some mutants. The data suggest a defect in a virion-associated protein, which prevents expression of early viral functions during lytic infection at the restrictive temperature. They also suggest a defect in an early viral gene required for initiation of cellular DNA synthesis. It seems that at least some of the information in the late region of the genomes codes for viral particle proteins. The data have elucidated some of the functions necessary at different stages of infection and transformation.

22. THE PROPERTIES OF A POLYOMA VIRUS MUTANT, TS3. (PP. 49-151)

Dulbecco R

Tumor Virus-Host Cell Interaction; Edited by A Kolber (New York, Plenum Press): 1975

A temperature-sensitive (ts) polyoma virus mutant, ts3, was described. Ts3 is temperature sensitive in transformation and lytic infection. Topoinhibition is low at low temperatures and higher at high temperatures, reflecting changes in serum requirement in crowded cell layers. The morphology of transformed cells changes with temperature, suggesting that continued expression of this mutation is needed for transformation. Several cell lines (BALBc/3T3 and PY6) were tested for virus yield or production of infectious viral DNA at 32 C and 39 C. Temperature-sensitivity proved to be cell-dependent. BALBc/3T3 cells were stimulated with serum at various times before and after infection. A lower ratio of yield was obtained when the serum was added 16-18 hr before infection, suggesting that the physiological state of the cell may influence the expression of the gene. The infectivity of purified viral DNA is not temperature-sensitive in BALBc/3T3 cells. Mutation is expressed very soon after infection. Ts3 will not complement with any other polyoma mutants in BALBc/3T3 cells at 39 C. This suggests that the mutation affects a virion protein. More research is needed to determine the nature of this mutation.

23. GENETIC STUDIES WITH TUMORIGENIC ADENOVIRUSES. I. ISOLATION OF CYTODIAL (CYT) MUTANTS OF ADENOVIRUS TYPE 12.

Takemori N, Riggs JL, Aldrich C

California State Dept. Pub. Health., Berkeley
Virology; 36(4):575-586 1968

Several cytodial mutants (designated cyt), identifiable by their different CPE's in human embryo kidney (HEK) cells, were isolated from stocks of the highly tumorigenic Huie and 1131 strains of adenovirus type 12 (AV-12). The frequency of these spontaneous mutants was approx. 0.001%; UV irradiation increased this frequency about 5-fold. Under conditions of mixed infection, the CPE of the parental stock (designated cyt+) was dominant. The cyt mutants, unlike the parental strains, did not transform newborn hamster kidney cells in vitro, and proved less tumorigenic than the resp parent

strain (with 1 exception). However, the cyt mutants enhanced the tumor-inducing effects of AV-7, AV-3, and 3 low-tumorigenic cyt+ strains of AV-12 (Bersiak, 97838 and 10516), though they did not cooperate with AV-2, AV-4 and AV-5. It is suggested that the cyt gene is responsible for both the tumor-inducing effects and the cyt+ CPE of AV-12.

24. A TEMPERATURE-SENSITIVE MUTANT OF ADENOVIRUS 31, DEFECTIVE IN VIRAL DEOXYRIBONUCLEIC ACID REPLICATION.

Suzuki E, Shimojo H

Natl. Inst. Hlth., Tokyo, Japan

Virology; 43(2):488-494 1971

The physiological activity of a temperature-sensitive mutant (ts 13) of adenovirus 31 was analyzed by means of DNA polymerase and thymidine kinase activity monitoring. The ts 13 mutant induced synthesis of T antigen, DNA polymerase and thymidine kinase but did not synthesize viral DNA and capsid protein at the nonpermissive temperature (39.5C). The temperature shift-down test showed that the function of a gene ts 13 must be expressed for viral DNA replication during the period of 16-24 hr postinoculation, whereas the temperature shift-up test showed that protein synthesized at low temperatures was either rapidly exhausted or inactivated at high temperatures. Although tumors were produced in hamsters by ts 13 at 37.5C, it is not known whether transformation of cells occurs at nonpermissive temperatures.

25. ONCOGENIC TRANSFORMATION OF HAMSTER EMBRYO CELLS IN VITRO BY ADENOVIRUS TYPE 5.

Williams JF

MRC Virology Unit, U. Glasgow, Scotland

Nature (Lond); 243:162-163 1973

To determine if adenovirus type 5 can transform permissive cells, secondary cultures of hamster embryo fibroblasts (HEF) were infected with one of the temperature sensitive (ts) mutants, ts1, ts4, or ts14, at the restrictive temperature which prevents cell killing caused by virus replication. For transformation, the infected cells were incubated at 38.5 C for two days, then subcultured by seeding at a density of either 4×10^5 or 10^6 cells per 50 mm dish. These subcultures were then cultivated at 38 C for up to 6 wk. Transformed foci first appeared approximately 4 wk after infection. The morphology of these transformed HEF cells was similar to that of rat embryo cells transformed by adenovirus type 5. A number of single foci were picked from cultures infected by each mutant and six lines established. Cells of all six lines retained the transformed morphology throughout many passages in vitro. The transformed cells could be superinfected by wild type virus at either temperature and by various ts mutants at the permissive temperature of 31 C. After five passages in vitro, cells of two of these lines were inoculated ip into 1-day old hamsters. Approximately 6 wk later, some of these animals had developed ip tumors, which were found to be transplantable and have been transplanted for up to six consecutive passages. In a few animals metastases to either lungs or liver were observed. The results indicate that the adenovirus type 5, until now considered to be a non-oncogenic adenovirus, is capable of oncogenic transformation of cells in vitro.

26. TEMPERATURE-SENSITIVE MUTANTS OF ADENOVIRUS TYPE 12 DEFECTIVE IN VIRAL DNA SYNTHESIS.

Ledinko N

Dept. Biology, Univ. Akron, Akron, Ohio

J Virol; 14(3):457-468 1974

Ten temperature-sensitive (ts) mutants of adenovirus type 12 which produce plaque at 31 but not a 38.5 C were isolated

after mutagenesis with nitrosoguanidine or nitrous acid. The mutants were classified into six separate complementation groups. DNA-DNA hybridizations showed that at 38.5 C the ts 401 and 406 mutants of groups B and E, respectively, synthesized less than 10% of the normal level of viral DNA. The two mutants were also defective in the production of late proteins at the nonpermissive temperature, as shown by fluorescent-antibody tests and analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The recombination frequency for the wild-type virus production was 17.7% for ts 401 x ts 406. Both mutants induced an increase in thymidine kinase activity at 38.5 C. Moreover, the two viral DNA-defective mutants shut off host DNA synthesis at the restrictive temperature. At 38.5 C ts virus 401 transformed 2-8 times more hamster cells than the wild-type virus, whereas ts virion 406 transformed at a frequency similar to the wild-type virus.

27. TRANSFORMATION CHARACTERISTICS OF TEMPERATURE-SENSITIVE MUTANTS OF TYPE 12 ADENOVIRUS.

Rubenstein FE, Ginsberg HS
Sch. Med., Univ. Pennsylvania, Philadelphia
Intervirology; 3(3):170-174 1974

Three temperature-sensitive mutants of type 12 adenovirus (ts 303, ts 307, and ts 333) were isolated after mutagenesis of wild-type (Wt) virus with 5-bromo-2-deoxyuridine (BUdR). Each of the three mutants effectively complemented the other two, the complementation index for each double infection being 490 times greater than the sum of the yield of the two single infections. The complementation yield of ts 307 x ts 303 was 15.1% of Wt virus. Wt type 12 transforms primary rat embryo cells in vitro; ts 303 transformed at a frequency closely similar to Wt, ts 333 had diminished transforming effect at 32 C and essentially did not transform at 39.5 C, and ts 307 transformed cells at a slightly higher frequency than Wt virus at 32 C but consistently produced 2-4 times more transformed foci at 39.5 C. The increased transformation reduced by ts 307 at the nonpermissive temperature may be the result of a lesion in a regulatory viral gene which normally suppresses transformation. The transformed foci in each case readily produced continuous cell lines whose cells contained intranuclear T antigen. Preliminary studies indicate that ts 307 is a DNA minus mutant which induces the synthesis of T antigen but does not produce capsid proteins; ts 303 does not produce immunologically active hexon protein; and ts 333 makes reduced amounts of viral DNA and capsid proteins and produces large plaques at the permissive temperature.

28. TRANSFORMATION OF HAMSTER EMBRYO AND HUMAN EMBRYO CELLS BY TEMPERATURE SENSITIVE MUTANTS OF HERPES SIMPLEX VIRUS TYPE 2.

Takahashi M, Yamanishi K
Res. Inst. Microb. Dis., Osaka Univ., Japan
Virology; 61(1):306-311 1974

The ability of 12 temperature-sensitive (ts) mutants of Herpes simplex virus type 2 (HSV-2) to transform hamster embryo (HaE) cells and human embryo (HuE) cells in vitro at the nonpermissive temperature (38.5 C) was studied. Three mutants (ts 4, 9, 35) gave rise to transformed HaE cells and one mutant (ts 9) induced transformation in HuE cells. All transformed cell lines have been passaged more than 50 times. The injection of transformed HaE cells into hamster resulted in the formation of tumors which were hard compared with

those induced by simian virus 40 (SV 40) for adenovirus and developed more slowly. Unlike SV 40 tumor-bearing animals or adeno-tumor-bearing animals, metastases of the tumor cells were frequently found in the lungs of the HSV-tumor-bearing hamsters. Cultured cells derived from these tumors were morphologically similar to the original transformed cells. No infectious HSV-2 has been recovered from the original HaE or HuE cells or the cultured tumor cells. Fluorescent antibody staining revealed distinct fluorescence in the cytoplasm of 5-10% of the transformed HaE and HuE cells; no fluorescence was seen in normal HaE or HuE cells or in simian adeno-SA7 transformed cells. Nor was any fluorescence detected when the cells were treated with preimmune, as opposed to postimmune, rabbit serum.

29. VIRAL GENE FUNCTIONS EXPRESSED AND DETECTED BY TEMPERATURE-SENSITIVE MUTANTS OF HERPES SIMPLEX VIRUS.

Benyesh-Melnick M, Schaffer PA, Courtney RJ, Esparza J, Kimura S
Dept. Virology and Epidemiology, Baylor Coll. Medicine, Houston, TX 77025
Cold Spring Harbor Symp Quant Biol; 39:731-746 1975

The expression of herpes simplex virus (HSV)-specific gene functions by 22 temperature-sensitive (ts) mutants of HSV-1 in 15 complementation groups and 8 ts mutants of HSV-2 in 7 complementation groups was studied at the nonpermissive temperature. Four cistrons of HSV-1 and three cistrons of HSV-2 with defects in viral DNA and DNA polymerase synthesis were identified. DNA- mutants of HSV-1 revealed a greater alteration in HSV polypeptide synthesis and viral assembly than DNA- mutants of HSV-2. Mutants with apparent defects in structural proteins were identified for both HSV-1 and HSV-2 by their increased thermostability. The provisional HSV-1 and HSV-2 linkage maps showed that the DNA- mutants were on the left-hand side of each map, and mutants with phenotypic properties similar to those of the wild-type virus were on the right-hand side. An early polypeptide of HSV (VP175, MW 175,000) accumulated in cells infected at the nonpermissive temperature with ts mutants of HSV-1 in complementation group B. Antiserum to this polypeptide discriminated between HSV-1 and HSV-2 by immunofluorescence. In contrast, type-specific gene functions of HSV-1 and HSV-2 were not demonstrated through intertypic complementation and recombination tests with heterologous mutant pairs, but mutually exchangeable or common gene functions were readily identified. Eight ts mutants of HSV-2 were used to detect functional HSV genes in hamster embryo cells transformed by HSV-2. Normal and SV40-transformed hamster cells failed to support the replication of the mutants at the nonpermissive temperature. However, the replication of two mutants, defective in late functions, was significantly enhanced in two independently derived HSV-2-transformed cell lines. Thus functional HSV genetic information was detected in HSV-2-transformed cells through the use of ts mutants. Moreover, it appears that the information present in both cell lines is not only specific but involves late HSV functions. (74 refs)

30. ONCOGENIC TRANSFORMATION OF PRIMARY HAMSTER CELLS BY HERPES SIMPLEX VIRUS TYPE 2 (HSV-2) AND AN HSV-2 TEMPERATURE-SENSITIVE MUTANT.

Kimura S, Flannery VL, Levy B, Schaffer PA
Baylor Coll. Med., Houston, Tex.
Int J Cancer; 15(5):786-798 1975

The induction, isolation, and preliminary characterization of six cell lines transformed by UV-irradiated Herpes simplex virus type 2 (HSV-2) and a temperature sensitive

mutant (ts mutant) of HSV-2 are described. Irradiated virus was adsorbed to 2-day-old hamster embryo fibroblasts for two hours at a multiplicity of 400 UV-inactivated particles per cell. Rabbit antiserum to HSV-2 was added to half the infected cultures before incubation. Foci of transformed cells were picked 40 days after infection and grown to confluency. In additional experiments, hamster embryo fibroblast monolayers were pretreated with 5-bromodeoxyuridine or UV irradiation prior to infection and cell foci were picked 34 days after infection. To obtain transformation with ts mutants of HSV, hamster embryo fibroblast monolayers were infected at a multiplicity of 5-10 plaque forming units per cell, and the virus was adsorbed for two hours at 37 C. Infected monolayers were incubated at the nonpermissive temperature, and cell foci were picked 30 days after infection. Cultures were tested for oncogenicity by injecting 0.1 ml (5 x 10⁵ cells) of each cell suspension sc into newborn hamsters which were observed for six months for tumor development. Two of 25 foci picked 40 days after infection could be subcultured indefinitely. Three cell lines were established from foci picked from pretreated infected cultures: two from 18 foci derived from UV-pretreated cultures, and one from 29 foci picked from bromodeoxyuridine-pretreated cultures. One of eight foci picked from cultures infected with a HSV-2 DNA-ts mutant survived more than 70 passages. Two additional mutant-transformed cell lines were established from 130 foci picked from cultures infected with a DNA+ HSV-1 ts mutant and a DNA+ HSV-2 ts mutant. All six transformed cell lines produced undifferentiated fibrosarcomas in newborn hamsters after, but not before, 33 passages. Four of six lines produced metastatic tumors in the lungs. Sera from tumor-bearing hamsters contained neutralizing antibody to HSV-2. HSV-specific cell-surface antigen was detected by immunofluorescence in all six of the lines established from primary tumors. Antibody to an early, HSV-specific, nonstructural polypeptide reacted by immunofluorescence with antigen(s) in fixed preparations of 3 of 6 tumor cell lines. No type-C virus group-specific-3 antigen was detected in either transformed or tumor-cell lines. It is suggested that the absence of such markers is presumptive evidence that type-C virus played no role in the transforming event, and that transformation was induced by HSV-2.

31. VARIATION IN SUSCEPTIBILITY OF DIFFERENT CELL TYPES TO TEMPERATURE-SENSITIVE HOST RANGE MUTANTS OF HERPES SIMPLEX VIRUS TYPE 2.

Koment RW, Rapp F
Milton S. Hershey Med. Cent. Pennsylvania State Univ.,
Hershey, PA
Virology; 64(1):164-169 1975

The ability to produce cytopathologic effects and to replicate at 39 C in various cell types were examined for four conditional lethal mutants (69, 74, 46, and 41) of Herpes simplex virus type 2 (HSV-2). Primary cell cultures were prepared from epithelioid cells (hamster, mouse and rabbit kidneys) and from fibroblastoid cells (hamster, mouse, rabbit and human lung tissues) and inoculated with virus at an infective multiplicity of 2. All cell types underwent transformation by HSV-1, and all four mutants replicated in all cell types at 33 C, producing total cytopathic effect. HSV-1 induced complete cytopathic effect in mouse embryo fibroblast cultures and replicated at 39 C. Although all mutants replicated in these cells at 39 C, no discernible cytopathic effect was observed. All epithelioid cells and human and rabbit fibroblastoid cells were permissive to virus replication at 39 C. The results indicate that these Herpes simplex virus type 1 mutants are temperature-sensitive and have host-range properties.

32. HOST RANGE TEMPERATURE-SENSITIVE MUTANTS OF HERPES SIMPLEX VIRUS TYPE 2.

Westmoreland D, Rapp F
Department of Microbiology, The Milton S. Hershey
Medical Center, The Pennsylvania State University, Col-
lege of Medicine, Hershey, Pennsylvania 17033
J Virol; 18(1):92-102 1976

Two small-plaque mutants of herpes simplex virus type 2 (HSV-2) (strain 333), whose growth at 39 C was blocked in certain cell types (cell-dependent temperature sensitivity), were compared with the parental virus in a number of biological assays. One mutant (no. 69) was found to produce a large number of morphologically normal, but noninfectious, particles; under nonpermissive conditions, these mutant particles were able to interfere with the replication of wild-type HSV-2. The other mutant (no. 74), which is known to belong to a different complementation group, appeared to direct little virus DNA synthesis, even at the permissive temperature. Progeny production and virus DNA synthesis in cells infected by mutant 74 were delayed in comparison with wild-type virus-infected cells. Both mutants were found to be more sensitive to UV irradiation than the parental virus; this was especially marked in the case of mutant 74. Moreover, this mutant was found to have a high transforming efficiency at much lower doses of irradiation than those needed to abolish the cytopathic effect of wildtype HSV-2.

B. Genetic Analysis

33. SPONTANEOUS SEGREGATION OF NONTRANSFORMING VIRUSES FROM CLONED SARCOMA VIRUSES.

Vogt PK
U. Washington Sch. Med., Seattle.
Virology; 46(3):939-946 1971

It is known that strains of Rous sarcoma virus (RSV) may be the source of nontransforming (NT) viruses. A study was performed to determine the incidence and levels of such NT viruses in various strains of helper independent avian sarcoma viruses, and to examine the possibility that the presence of NT viruses is the result of passage through group-specific antigen (gs) positive chicken cells. It was determined by seeding and challenging techniques that NT virus could be detected in the presence of excess sarcoma virus when cloned on gs-negative chick embryo fibroblasts. NT virus was then isolated in six out of seven of the clones; NT virus occurrence was between 4 and 17% of the sarcoma virus concentration. It was found that the NT virus had plating efficiencies and host ranges identical to the sarcoma viruses; in addition, the envelopes of both NT and sarcoma viruses were the same. Thus, NT viruses are probably spontaneous mutants occurring regularly and inevitably during sarcoma virus replication; the emergence of such viruses, therefore, does not require the induction of a resident leukemia virus.

34. GENETIC RECOMBINANTS AND HETEROZYGOTES DERIVED FROM ENDOGENOUS AND EXOGENOUS AVIAN RNA TUMOR VIRUSES.

Weiss RA, Mason WS, Vogt PK
U. Southern California Sch. Med., Los Angeles
Virology; 52(2):535-552 1973

A selective technique based on infective centers is described for isolating host range recombinants of avian RNA tumor viruses. The term recombinant is used to indicate any virus which carries genes derived from two parents, without distinguishing between reassortment of independent genome segments and recombination within one segment. This method was exploited to obtain recombinants between the genome of chick helper factor (chf) and nondefective strains of Rous

sarcoma virus (RSV), including temperature-sensitive mutants. Recombinants of chf with the defective Bryan strain RSV could not be isolated. A majority of nondefective RSV particles selected as carrying the chf host range also carried the parental RSV host range; i.e., were of dual host range, but the progeny segregated into parental or recombinant genotypes. These observations suggested that the particles were at least partially diploid or polyploid and represented unstable 'heterozygotes.' Genotypic mixing was not evident in chf-negative cells which contain viral DNA but not viral RNA, suggesting that genetic reassortment occurs among RNA molecules. A model is proposed in which reassortment of independent genome segments may be converted into stable recombinants following provirus formation in the next replicative cycle.

35. GENETIC RECOMBINATION AMONG TEMPERATURE-SENSITIVE MUTANTS OF ROUS SARCOMA VIRUS.

Wyke JA, Bell JG, Beamand JA

No Affiliation Given

Tumor Viruses Cold Spring Harbor Symposia on Quantitative Biology; Cold Spring Harbor Lab. (Cold Spring Harbor, NY): Vol 39, pp 897-905; 1975

Genetic recombination of Prague Rous sarcoma virus (RSV) was studied, with temperature sensitive (ts) mutations (ts LA 22, 35, and 335) in both initiation and maintenance of transformation as markers. The progeny of a single cycle of mixed infection contained no recombinants, but yielded heterozygous particles or viral clumps. On subsequent cycles of infection some of these heterozygotes/clumps persisted, they also segregated recombinant viruses. Some of the markers in these recombinants showed evidence of linkage and thus probably recombined by intramolecular exchanges. These results demonstrate that recombination at different frequencies can occur among these mutants. Studies of transformation defective (td) mutants (the class most frequently isolated from stock virus) showed that recombination occurred at a level sufficient to explain co-operative transformation by these viruses. Furthermore, this genetic recombination was probably a necessary prerequisite for cooperative transformation since complementation between the mutants is absent or inefficient. The simplest explanation for this apparent lack of complementation is that the td ts mutants are all derived by lesions in the same cistron.

36. THE A SUBUNIT IN THE RNA OF TRANSFORMING AVIAN TUMOR VIRUSES: I. OCCURRENCE IN DIFFERENT VIRUS STRAINS. II. SPONTANEOUS LOSS RESULTING IN NONTRANSFORMING VARIANTS.

Martin GS, Duesberg PH

Molecular Biology and Virus Lab. Univ. California, Berkeley, CA 94720

Virology; 47(2):494-497 1972

The RNA structure of two strains of Rous sarcoma virus (RSV) was examined to clarify whether the (a) subunit of the viral RNA contains information necessary for transformation of avian cells or whether it is an RNA species synthesized only in transformed cells, i.e., whether it is a cause or a consequence of the transformed state. The mutant strains were T5, a temperature-sensitive mutant of the Schmidt-Ruppin strain of subgroup A (SR-RSV-A) and the Bryan strain (B-RSV). T5 grows at the same rate as its wild type parent at 41°C but cannot transform fibroblasts at that temperature. The virus T5 released at 41°C can transform cells at 36°C; hence, the genetic information needed for transformation must be present in T5 produced at 41°C. Heat-associated T5 RNA comigrated with heat-dissociated RNA from wild-type virus in polyacrylamide gels. Thus, it is concluded that synthesis of the (a) subunit of

RSV RNA is not dependent on the transformation of the cell. On the other hand, B-RSV appears to contain very little (a) subunit. Since the majority of B-RSV RNA is derived from particles which are genetically unable to transform cells, the presence of the (a) subunit is correlated with the ability of the virus to transform cells rather than with the physiological state (transformed or not transformed) of the cells from which the particles were released. It was also demonstrated that the (a) subunit could be lost from SR-RSV-A viruses spontaneously, giving rise to nontransforming particles. (21 Refs)

37. TEMPERATURE-SENSITIVE MUTANTS OF AVIAN SARCOMA VIRUSES: GENETIC RECOMBINATION WITH WILD TYPE SARCOMA VIRUS AND PHYSIOLOGICAL ANALYSIS OF MULTIPLE MUTANTS.

Hunter E, Vogt PK

Univ. Southern California Sch. Medicine, 2025 Zornd Ave., Los Angeles, CA 90033

Virology; 69(1):23-34 1976

Genetic recombination experiments were done to analyze the genotype of two coordinate temperature sensitive (ts) mutants of the avian sarcoma viruses, LA334 and LA338. Recombination between Prague strain B of Rous sarcoma virus (PR-B) and LA334 resulted in progeny consistent with the mutant having two lesions, one affecting the maintenance of transformation, the other replication of infectious virus. LA338 appears to possess at least three mutations; one is similar to that in LA334 and affects the maintenance of transformation; a second prevents the synthesis of infectious virus at the restrictive temperature; the third affects an early function necessary to initiate transformation. The early defect can be complemented by and is lost on recombination with leukemia viruses. Temperature shift experiments confirmed that the defective replication functions in LA334 and LA338 are late and reversible. They can both be complemented by leukemia viruses, but appear to affect different stages in replication. In fluorescent antibody studies at the nonpermissive temperature, LA334-infected cells contained normal levels of the major viral structural polypeptides; LA338-infected cells contained reduced amounts. High frequency recombination was observed between host range and transformation markers. Of the 12 recombinant clones of LA334, 10 recombined in the part of the genome containing the replication defect, but retained the host range genes of the mutant. Apparently the defect of LA334 is not in the host-range genes.

38. RECOMBINATION BETWEEN A TEMPERATURE-SENSITIVE MUTANT AND A DELETION MUTANT OF ROUS SARCOMA VIRUS.

Kawai S, Hanafusa H

The Rockefeller University, New York, New York 10021

J Virol; 19(2):389-397 1976

Cells doubly infected with two mutants of the Schmidt-Ruppin strain of Rous sarcoma virus (RSV), ts68, which is temperature sensitive for cell transformation (src^{ts}), and a deletion mutant, N8, which is deficient in the envelope glycoprotein (env-), produced a recombinant which carried the defects of both parents. The frequency of formation of such a recombinant was exceptionally high and made up 45 to 55% of the progeny carrying the src^{ts} marker. By contrast, the reciprocal recombinant, which is wild type in transformation (src⁺) and contains the subgroup A envelope glycoprotein (env⁺), was almost undetectable. This remarkable difference in the frequency of the formation of the two possible recombinants suggests that a unique mechanism may be involved in the genetic interaction of the two virus genomes, one of which has a large deletion. When an RNA-dependent DNA

polymerase-negative variant of the N8 (N8alpha) was crossed with ts68, the majority (60 to 80%) of the src**tsenv-recombinants also became deficient in the polymerase. Cells infected by the src**tsenv- recombinant were morphologically normal at the nonpermissive temperature (41 degrees C) and susceptible to all subgroups of RSV. The rate by which the wild-type RSV transformed the recombinant-preinfected cells was indistinguishable from that of transformation of uninfected chicken cells by the same wild-type virus. This indicates that no detectable interference exists at postpenetration stages between the preinfected and superinfecting virus genomes and confirms that the expression of the transformed state is dominant over the suppressed state. (Author Abstract)

39. IDENTIFICATION OF INFECTIOUS VIRIONS WHICH ARE VESICULAR STOMATITIS VIRUS PSEUDOTYPES OF MURINE TYPE C VIRUS.

Livingston DM, Howard T, Spence C
Div. Medical Oncology, Sidney Farber Cancer Center,
Boston, MA
Virology; 70(2):432-439 1976

Superinfection of a sarcoma-leukemia type C virus producing cell with a temperature-sensitive vesicular stomatitis virus mutant (VSV-ts1026) produced pseudotype virions containing the type C genome and one or more VSV-1026 envelope proteins. The type C virus was a focus-forming Rauscher pseudotype of Kirsten murine sarcoma virus KiMSV (R-MuLV). Infection of RRK-KiNRK cells a Kirsten MSV-transformed nonproducer line infected with and producing the KiMSV (R-MuLB) focus-forming virus with VSV-ts1026 produced virions whose focus-forming activity was resistant to goat anti-Rauscher and VSV-New Jersey neutralizing antibodies. However, the focus-forming activity of these virions was dramatically reduced by anti-VSV-Indiana neutralizing antibody. Since VSV-ts1026 is in the Indiana serotype class and VSV-Ind and VSV-NJ are antigenically distinct viral subclasses with no immunological cross-reactivity, the authors conclude that the new focus-forming pseudotype virus contains at least one envelope antigen specifically donated by VSV-ts1026. The data suggest that the new VSV pseudotype virus has a functionally intact type C viral genome, since: (1) foci produced by pseudotype and KiMSV (R-MuLV) virions are identical and (2) VSV pseudotype-produced foci contain rescuable sarcoma viruses that produce normal foci following transmission to NRK cells. (9 refs)

40. THE SEMIAUTONOMOUS REPLICON: A MOLECULAR MODEL FOR THE ONCOGENICITY OF SV40. (PP. 17-24)

Martin RG, Chou JY, Avila J, Saral R
Tumor Viruses Cold Spring Harbor Symposia on
Quantitative Biology; Cold Spring Harbor Lab. (Cold
Spring Harbor, N.Y.); vol. 39, 1975

A large number of temperature-sensitive (ts) mutants of simian virus 40 (SV40) were isolated and characterized. Their behavior in permissive cells and studies of transformation and of the transformed cell lines induced by the mutants are summarized. A model is presented to account for the oncogenicity of SV40. Sixty-eight ts SV40 mutants were classified into five groups (A, B, C, BC, and D) on the basis of their behavior in complementation tests, and their transformation properties were characterized with use of secondary adult Chinese hamster lung cells. It was suggested that: (a) the expression of A function by an integrated SV40 genome in a nonpermissive or semipermissive cell line leads to aberrant initiation of host DNA synthesis; (b) that the majority of parameters generally associated with transformation are normal properties of cells in the S, G2, or M phase of growth; and (c) that the expression

of A function is determined by its site of integration and therefore is not fully autonomous. This model can explain different levels of SV40 transformation, but only if a hierarchy of phenotypes exists. The expression of the viral initiator would determine how long a cell stays in G0, and this, in turn, would determine to what extent "transformation" properties are manifest.

41. SIMIAN VIRUS 40 MUTANTS CARRYING TWO TEMPERATURE-SENSITIVE MUTATIONS.

Chou JY
Section on Developmental Enzymology, Laboratory of
Biomedical Sciences, National Institute of Child Health
and Human Development, Bethesda, Maryland 20014
J Virol; 20(1):39-44 1976

Five temperature-sensitive mutants of simian virus 40 containing two temperature-sensitive mutations were isolated. The double mutant of the A and D complementation groups, like the D mutants, failed to complement by conventional complementation analysis and did not induce host DNA synthesis at 40 degrees C. However, under conditions that suppressed the D defect, the A:D double mutant expressed only the A defect. Thus, viral DNA replication dropped rapidly after this mutant was shifted from permissive to restrictive temperatures. The A:D double mutant failed to transform at the restrictive temperature when subconfluent Chinese hamster lung monolayers were used. Double mutants of A:B, A:C, and A:BC complementation groups, like their A parent, were defective in viral DNA replication, in the induction of host DNA synthesis, and in the transformation of secondary Chinese hamster lung cells at the nonpermissive temperature. (Author Abstract)

42. HEAT-STABLE VARIANT OF HUMAN ADENOVIRUS TYPE 5: CHARACTERIZATION AND USE IN THREE-FACTOR CROSSES.

Young CS, Williams JF
Inst. Virol., Glasgow, Scotland
J Virol; 15(5):1168-1175 1975

The isolation and partial characterization of a heat-stable (hs) variant of human adenovirus type 5 (Ad5) is reported. The hs mutant was isolated from the temperature-sensitive mutant (ts1), the heat stability of which is similar to that of the wild type. Mutant ts1 was inactivated at 52 C and a portion of the surviving fraction was seeded on HeLa cells to obtain a higher-titer stock. A portion of this stock was then inactivated at 52 C and a second high-titer stock was obtained. Plaques isolated 5, 10, and 15 min after heat inactivation of this second stock were tested for heat stability at 52 C. A putative heat-stable mutant was thus obtained. The variant was genetically stable, both through vegetative viral passage and through recombination into other genetic backgrounds; this suggests that it arose from a single mutation. Three-factor crosses using this mutant in conjunction with ts mutants further suggest that the hs mutation lies near the left hand end of the genetic map. The mutant was used to demonstrate the production of reciprocal recombinants in two-factor crosses. The mutational lesion is unknown, but phenotypic mixing occurs in hs x hs+ infections, which suggests that it lies in a gene specifying a virion structural protein. The following phenotypic characteristics were identical in the hs and wild-type hs+ viruses: ability to transform rat embryo cells; ability to induce interferon on chick embryo fibroblasts; frequency of ts+ recombinants in crosses of the general types tsx x tsy, tsx-hs x tsy, tsx x tsy-hs, and tsx-hs x tsy-hs, inactivation by neutralizing antiserum to Ad5, stability on storage at -20 and -70 C, ability to form infection centers, particle to PFU ratios, and yields of virus at 32, 37, and 38 C. The hs marker can be used in three-factor crosses to aid in the production of an unambiguous genetic map of Ad5.

43. GENETIC ANALYSIS OF HUMAN ADENOVIRUS TYPE 5 IN PERMISSIVE AND NONPERMISSIVE CELLS. (PP. 427-437)

Williams JF, Young CC, Austin PE

Tumor Viruses Cold Spring Harbor Symposia on Quantitative Biology; Cold Spring Harbor Lab. (Cold Spring Harbor, NY): vol. 39, 1975

Recent results concerning the genetic analysis of human adenovirus type 5 (Ad5) using temperature-sensitive (ts) mutants, and results relating the interaction of ts mutants with other viruses and to the ability of mutants to transform rat embryo cells are presented. An upper limit of about 10^{-4} was estimated for most of the ts mutants. On the basis of complementation tests, 51 mutants were classified into 5 functional groups; in most cases complementation was either positive or negative. Complementary crosses between Ad5 and Ad12 produced viruses possessing antigenic determinants from both parental types; this double antigenicity was due to phenotypic mixing, not genetic recombination. Most Ad5 mutants were not enhanced at all by simian virus 40 (SV40) in complementation tests, but mutants of five complementation groups showed varying degrees of enhancement. A tentative ts mutant genetic map was constructed, and the following conclusions were drawn from the data obtained: stable recombinants are formed in crosses between different mutants; recombination frequencies between different ts mutants are not random, and markers can be ordered in a basic additive sequence. Noncomplementary mutants show very low recombination frequencies; mutants showing low complementation indices also show low recombination frequencies; and, mutants that complement well show higher recombination frequencies. The ability of Ad5 (wild-type and ts mutants) to transform rat embryo cells was investigated. Recent evidence indicates that the genes necessary for maintaining the transformed state of rat cells must reside in a 14% segment located at the left end of the genome.

44. MUTANT OF HERPES SIMPLEX VIRUS TYPE 1 CONDITIONALLY ABLE TO TRANSFORM THYMIDINE KINASELESS L CELLS TO A TK+ PHENOTYPE.

Hughes RG, Munyon WH

Department of Medical Viral Oncology, Roswell Park Memorial Institute, Buffalo, New York 14263
J Virol; 18(6):867-872 1976

After nitrous acid mutagenesis of herpes simplex virus type 1 (HSV-1), a mutant, 1093, was isolated which, during productive infection, induced very low levels of thymidine kinase (tk). The mutant virus was found, after UV irradiation, to be unable to transform L cells lacking tk (Ltk⁻) to a tk⁺ phenotype as characterized by growth of the cells in a modified HAT-selective medium containing 1.6×10^{-5} M thymidine. Cells transformed by wild-type virus grew vigorously under the same conditions. The mutant was able to transform Ltk⁻ cells if the medium contained 10^{-3} M thymidine. These transformed cells maintained their conditional character and would not grow in low concentrations of thymidine in selective medium. Therefore, this mutant is conditional on the thymidine concentration in the selection medium in its ability to transform Ltk⁻ cells to a tk⁺ phenotype. The conditionally transformed cells could be supertransformed with wild-type UV-irradiated HSV-1 to a phenotype which would grow in low-thymidine selective medium. The frequency of supertransformation closely approximated the frequency of transformation of Ltk⁻ cells by wild-type virus. Supertransformation at high frequency could not be effected by mutant 1093 or the tk⁻ mutant B2006. These results indicate that the presence of HSV-1 genetic information in HSV-1-transformed cells does not preclude the

acquisition by these cells of at least one additional HSV-1 gene, that for tk. (Author Abstract)

C. Gene Mapping and Genome Structure of Virus Mutants

45. DIFFERENCES BETWEEN THE RIBONUCLEIC ACIDS OF TRANSFORMING AND NON-TRANSFORMING AVIAN TUMOR VIRUSES (MEETING ABSTRACT).

Duesberg PH, Vogt PK

Dept. Molec. Biol., Univ. California, Berkeley

Proc Natl Acad Sci USA; 67(4):1673-1680 1970

Differences in the RNAs of a transforming avian sarcoma virus B77 and its nontransforming derivatives both in their native forms and after heat dissociation into subunits are reported; the nontransforming viruses were isolated after UV irradiation of B77. The viruses were grown in suspension cultures of myeloblasts from leukemic chickens, and viral RNA was labeled with 3H-uridine and 32P-H3P04. The RNAs of the nontransforming virus were found to migrate slightly faster than RNA of B77 with gel electrophoresis. Electropherograms of the heat-dissociated RNAs demonstrated both classes of RNA subunits a and b in B77, but in 2 of the non transforming viruses, NT B77 clone 5 and NT B77 clone, the class a subunit was absent. The absence of class a RNA may be related to the inability of these viruses to transform chick embryo fibroblasts in tissue culture, or its presence may be merely the consequence of the transformed state of the cell.

46. THE 60-70S RNA OF AVIAN SARCOMA AND LEUKOSIS VIRUSES - DISTRIBUTION OF CLASS A AND B SUBUNITS.

Duesberg PH, Vogt PK, Martin GS

Dept. Mol. Biol., Univ. California, Berkeley

Bibl Haematol; (39):462-473 1973

Two electrophoretically separable classes of subunits, a and b, were found after heat dissociation of the 60-70S RNA obtained from several avian sarcoma viruses Schmidt-Ruppin Rous sarcoma virus (SR RSV-A), Prague strain RSV, RSV (RAV-O), virus B77. Avian leukosis viruses (avian myeloblastosis virus, Carr-Zilber associated virus) yielded only class b subunits. The difference between sarcoma and leukosis viruses was not affected by the transformed or normal state of the host cell; e.g., class a subunits were present in progeny particles of a temperature-sensitive SR RSV-A mutant grown at the nonpermissive temperature for transformation. On the other hand, leukosis viruses released from sarcoma cells did not acquire class a subunits; e.g., fibroblast cultures transformed by the Bryan high-titer strain of RSV synthesized an excess of nonfocus-forming helper virus, but viral harvest contained few if any class a subunits. A similar preponderance of nonfocus-forming virus may also explain the lack of class a subunits in avian leukosis virus strain MC29, which is capable of forming neoplastic foci in fibroblast cultures. A connection between the ability to form foci in fibroblast cultures and the presence of class a subunits was further suggested by the observation that cloned stocks of SR RSV-A segregate nonfocus-forming virus that, together with its transforming potential, has lost class a subunits. These results are in agreement with the hypothesis that class a and b subunits of avian tumor virus RNA represent functionally specialized segments of the viral genome which may replicate independently.

47. NUCLEOTIDE SEQUENCE RELATIONSHIPS OF AVIAN RNA TUMOR VIRUSES: MEASUREMENT OF THE DELETION IN A TRANSFORMATION-DEFECTIVE MUTANT OF ROUS SARCOMA VIRUS.

Neiman PE, Wright SE, McMillin C, MacDonnell D
U. Washington Sch. Med., Seattle
J Virol; 13(4):837-846 1974

Stocks of cloned helper-independent Rous sarcoma virus (RSV) spontaneously segregate transformation-defective (td) mutants that appear to have an RNA genome composed of smaller subunits than those of the parent virus. Differential hybridization and competitive hybridization techniques involving reactions between viral RNA and proviral sequences in host cell DNA (under conditions of initial DNA excess) were used to measure the extent of the deletion in a td mutant of the Prague strain (Pr) of RSV (PrRSV-C). Viral 60-70S RNA sequences labeled to 1 to 5 x 10⁷ cpm/microg with 125I were characterized with respect to their properties in hybridization reactions and were used to reinforce data obtained with 3H-RNA of lower specific activity. About 13 plus or minus 3% of the PrRSV-C sequences forming hybrids with DNA from virus-induced sarcomas appeared to be deleted from the genome of td PrRSV-C. Studies comparing the hybridization of RNA from PrRSV-C and td PrRSV-C with RSV-related sequences in normal cells, and competition experiments with RNA from the endogenous chicken oncornavirus Rous-associated virus type 0 (RAV-0) showed most if not all, of the RNA sequences of PrRSV-C deleted from its transformation-defective mutant are not represented in normal chicken DNA. Competition studies with a leukosis virus, RAV-7, indicated that this virus also lacks a genome segment of about the same size as the deletion in the td mutant. Finally, the genome of all three 'exogenous' viruses lacked a small segment (about 12%) of sequences present in the endogenous provirus of RAV-0.

48. PROPERTIES AND LOCATION OF POLY(A) IN ROUS SARCOMA VIRUS RNA.

Wang LH, Duesberg P
Dept. Mol. Biol., U. California, Berkeley
J Virol; 14(6):1515-1529 1974

The poly(A) sequence of 30-40S Rous sarcoma virus RNA, prepared by digestion of the RNA with RNase T1, showed a rather homogenous electrophoretic distribution in formamide-polyacrylamide gels. Its size was estimated at about 200 AMP residues. The poly(A) appears to be located at or near the 3' end of the 30-40S RNA because (i) it contains one adenosine/180 AMP residues, and because (ii) incubation of 30-40S RNA with bacterial RNase H in the presence of poly(dT) removed its poly(A) without significantly affecting its hydrodynamic or electrophoretic properties in denaturing solvents. The viral 60-70S RNA complex was found to consist of 30-40S subunits both with (65%) and without (approximately 30%) poly(A). The heteropolymeric sequences of these two species of 30-40S subunits have the same RNase T1-resistant oligonucleotide composition. Some, perhaps all, RNase T1-resistant oligonucleotides of 30-40S Rous sarcoma virus RNA appear to have a unique location relative to the poly(A) sequence since the complexity of poly(A)-tagged fragments of 30-40S RNA decreased with decreasing size of the fragment. Two RNase T1-resistant oligonucleotides which distinguish sarcoma virus Prague B RNA from that of transformation-defective deletion mutant of the same virus appear to be associated with an 11S poly(A)-tagged fragment of Prague B RNA. Thus, RNA sequences concerned with cell transformation seem to be located within 5-10% of the 3' terminus of Prague B RNA.

49. THE LOCATION OF THE DELETION IN TRANSFORMATION-DEFECTIVE ROUS SARCOMA VIRUS RNA.

Perez-Bercoff R, Billeter MA
Institut für Molekularbiologie I, Universität Zürich,
Hönggerberg, CH-8049 Zürich
Experientia; 32(6):806 1976

Transformation defective (td) mutants of Rous sarcoma virus RNA lack an RNA segment, as shown by the decreased chain length of their RNA (Martin and Duesberg, Virology 47, 494, 1972) and the absence of 3 characteristic large T1 oligonucleotides (Coffin and Billeter, J Mol Biol, in press). Since these oligonucleotides are located close to the poly A segment, the question arose as to whether the deletions were close to the 3' end, but internal, or whether the whole RNA region immediately adjacent to the poly A was eliminated. (³²P) RNA from td and wild type Prague B RSV was partially digested with T1 RNase, and poly A-containing fragments were isolated by poly U-Sephadex chromatography. These were between 150 and 300 nucleotides in length as revealed by polyacrylamide gel electrophoresis, and contained on the average 80% AMP residues. The T1 and pancreatic fingerprints of the fragments derived from both viruses were identical, suggesting that a region of at least 100 nucleotides immediately adjoining the poly A segment was not deleted in transformation defective virus, in agreement with conclusions by Wang et al. (J Virol 16, 1051, 1975). (Author Abstract)

50. RNA OF REPLICATION-DEFECTIVE STRAINS OF ROUS SARCOMA VIRUS (MEETING ABSTRACT).

Duesberg PH, Kawai S, Wang LH, Vogt PK
Dept. Molec. Biol., Virus Lab., Univ. of California,
Berkeley, CA
Proc Natl Acad Sci USA; 72(4):1569-1573 1975

The RNA of a replication-defective (rd) mutant (isolated from nondefective (nd) Schmidt-Ruppin Rous sarcoma virus of subgroup A (SR-A) and designated SR-N8) was compared to the RNAs of SR-A, transformation-defective (td) SR-A, and to rd Bryan Rous sarcoma virus, RSV(-) SR-N8-transformed chick embryo fibroblasts were infected with td SR-A to form SR-N8 pseudotypes. The RNAs of all species were subjected to electrophoresis in aqueous and formamide polyacrylamide gels. The 30-40S RNA of SR-N8 migrated faster than the 30-40S RNA from SR-A or RSV(-) but did show some asymmetry when compared to standards. The 30-40S RNA of RSV(-) had the same mobility as RAV-2 RNA standard. Td SR-A had a higher mobility than SR-A, but lower than that of RAV-2. The difference in molecular mass between the 30S and 40S RNAs of SR-A and SR-N8 was 0.75 x 10⁶ daltons. Using fingerprint analysis, a homologous oligonucleotide spot was found in the pattern of SR-A, for every large SR-N8 RNA. The td SR-A contained all but two oligonucleotide spots found in SR-A. Comparing the fingerprint patterns of SR-A SR-N8 and td SR-A suggests that the deletions affecting transforming ability and those affecting a replicative function involve different regions of the viral RNA. Eleven of 13 large RNase T1 resistant oligonucleotides of SR-N8 have a counterpart in SR-A. The complexity of SR-A RNA was calculated at 1.17 x 10⁴ nucleotides and SR-N8 at 0.93 x 10⁴ nucleotides; the average nucleotide of the two RNAs had a molecular mass of 323 daltons. It is concluded that RNA of SR-N8 is a deletion of SR-A.

51. LOCATION OF ENVELOPE-SPECIFIC AND SARCOMA-SPECIFIC OLIGONUCLEOTIDES ON RNA OF SCHMIDT-RUPPIN ROUS SARCOMA VIRUS (MEETING ABSTRACT).

Wang LH, Duesberg PH, Kawai S, Hanafusa H
Dept. Molecular Biology and Virus Lab., Univ. California,
Berkeley, Calif. 94720
Proc Natl Acad Sci USA; 73(2):447-451 1976

A chemical method to map viral genes was applied to various avian sarcoma viruses. Envelope-specific and sarcoma-specific nucleotide sequences were located within the 10,000 nucleotides of the RNA of nondefective Schmidt-Ruppin Rous sarcoma virus (nd SR). About 30 RNase-T1 resistant oligonucleotides were ordered relative to the 3'-poly(A) terminus of the RNA, to construct an oligonucleotide map of the nd SR RNA. A cluster of seven envelope-specific oligonucleotides, identified by their absence from an otherwise very similar oligonucleotide map of an envelope-defective deletion mutant (lacking the major viral glycoprotein), mapped at a distance of 2800-5000 nucleotides from the poly(A) end of nd SR RNA. A cluster of two sarcoma-specific oligonucleotides, identified by their absence from an otherwise nearly identical oligonucleotide map of a transformation-defective deletion mutant, mapped at a distance of 1000-2000 nucleotides from the poly(A) end of nd SR RNA. The oligonucleotide maps of nd SR and of the two deletion mutants were the same from the poly(A) end up to 650 nucleotides and included one terminal oligonucleotide, termed C (found in all avian tumor viruses tested). A postulated gene order consistent with this data suggests that sarcoma-specific nucleotide sequences map between envelope-specific nucleotide sequences and the poly(A) end of the RNA.

52. SEQUENCES AND FUNCTIONS OF ROUS SARCOMA VIRUS RNA.

Duesberg PH, Wang LH, Beemon K, Kawai S, Hanafusa H
Virus Lab. and Dept. Molecular Biology, Univ. California,
Berkeley, CA 94720
Haematol Bluttransfus; 19:327-340 1976

The genetic elements of avian tumor virus RNA, which has a molecular wt of about 3×10^6 daltons and a poly(A) sequence at the 3' end, were mapped. About 30 RNase T1-resistant oligonucleotides were ordered relative to the 3'-poly(A) terminus of the RNA in order to construct an oligonucleotide map of viral RNAs. A cluster of seven envelope-specific oligonucleotides, which were identified by their absence from the otherwise very similar oligonucleotide map of an envelope-defective deletion mutant (which lacks the major viral glycoprotein) were mapped at a distance of $0.9-1.6 \times 10^6$ daltons from the poly(A) end of sarcoma virus RNA. Another cluster of three sarcoma gene-specific oligonucleotides, which were identified by their absence from the otherwise nearly identical oligonucleotide map of a transformation-defective deletion mutant were mapped at a distance of $0.2-0.6 \times 10^6$ daltons from the poly(A) end of sarcoma virus RNA. The oligonucleotide maps of sarcoma viruses and of related deletion mutants were the same from the poly(A) end up to 0.2×10^6 daltons and included one terminal oligonucleotide, termed C, which is found in all avian tumor viruses tested so far. Preliminary mapping experiments ordering the sarcoma gene-specific and envelope gene-specific oligonucleotides of recombinants, selected for sarcoma and envelope genes of different parents, agreed with those obtained by comparing maps of wild type viruses and deletion mutants. A partial genetic map consistent with these results suggests that the sarcoma gene maps between the envelope gene and the 3'-poly(A) end of viral RNA. The map reads: poly(A)-sarcoma gene-envelope gene-(viral DNA polymerase gene and viral group-specific antigen gene). (19 Refs)

53. PURIFICATION OF DNA COMPLEMENTARY TO NUCLEOTIDE SEQUENCES REQUIRED FOR NEOPLASTIC TRANSFORMATION OF FIBROBLASTS BY AVIAN SARCOMA VIRUSES.

Stehelin D, Guntaka RV, Varmus HE, Bishop JM
I.R.S.C., B.P. 8, 94800-Villejuif, France
J Mol Biol; 101(3):349-365 1976

Radioactive DNA complementary to nucleotide sequences representing a portion of the viral gene(s) required for transformation of fibroblasts (cDNA-sarc) by avian sarcoma virus (ASV) was prepared. In addition, some physical and chemical properties of cDNA sarc are presented, and the distribution of nucleotide sequences homologous to cDNA sarc among various RNA tumor virus strains is explored. Single-stranded DNA complementary to the AS virus genome was annealed to 70S RNA from a transformation-defective variant; the DNA that failed to anneal, representing viral nucleotide sequences absent from the genome of the deletion mutant, was separated from RNA/DNA hybrids by chromatography on hydroxyapatite with a shallow gradient of sodium phosphate and supplemented with 0.6 M NaCl to stabilize the RNA/DNA hybrids. cDNA sarc was a relatively uniform transcript from 16% of the nucleotide sequences in the genome of Prague strain-subgroup C ASV, and its genetic complexity was about 1,600 nucleotides, sufficient to represent an entire cistron. The genomes of three independent isolates of AS viruses shared nucleotide sequences closely related to cDNA sarc, whereas the sequences were absent from transformation-defective mutants of ASV, several avian leukemia viruses, a nonpathogenic endogenous virus of chickens (Rous-associated virus-O), sarcoma-leukosis viruses of mice and cats, and mouse mammary tumor virus. The authors conclude that the transforming gene(s) of all ASV have closely related or common genetic lineages distinct from the transforming genes in sarcoma viruses of other species. The results confirm previous reports that the transformation-defective variants of AS viruses are mutants with identical regions deleted from each subunit of a polyploid genome. (41 refs)

54. DNA RELATED TO THE TRANSFORMING GENE(S) OF AVIAN SARCOMA VIRUSES IS PRESENT IN NORMAL AVIAN DNA.

Stehelin D, Varmus HE, Bishop JM, Vogt PK
Dept. Microbiology, Univ. California, San Francisco, CA
94143

Nature (Lond); 260(5547):170-173 1976

DNA complementary to the transforming DNA of the Prague-C strain of avian sarcoma virus (ASV) has been hybridized to DNA isolated from normal non-transformed avian or mammalian sources. The transformation-specific DNA (cDNAsarc), which represented 16% of the total viral genome, corresponded to that portion of the genome of transformation-positive virus missing in transformation-defective deletion mutants. The extent of reaction of 0.32 ng/ml of the transforming DNA with 8 mg/ml of denatured avian or animal DNA was: 52% for chick embryo DNA, 46% for quail embryo DNA, 48% for adult turkey liver DNA, 45% for duck embryo DNA, 24% for 22-day-old emu liver and heart DNA, and less than 2% for calf thymus or mouse neonate DNA. The melting temperatures of formed hybrids (duplexes between cDNAsarc and normal DNA) were 77 C, 74 C, 71 C, and 70 C for the chick, quail, turkey, duck and emu DNA duplexes, respectively, and 81 C after hybridization to the DNA of rat cells containing transforming ASV provirus. Thus the DNA of normal chicken cells contains nucleotide sequences closely related to at least a portion of the transforming gene(s) of ASV; similar sequences are widely distributed among the DNA of avian species with a divergence

that roughly corresponds to the phylogenetic distances between species. Mammalian cells do not contain similar sequences. It is suggested that part or all of the transforming gene(s) of ASV may have been derived from the chicken genome or from a species closely related to chicken, either by a process akin to transduction or by other events.

55. DETECTION OF NUCLEOTIDE SEQUENCES ASSOCIATED WITH TRANSFORMATION BY AVIAN SARCOMA VIRUSES.

Stehelin D, Varmus HE, Bishop JM
Institut de Recherches Scientifiques sur le Cancer, BP No. 8, F-94800 Villejuif, France
Bibl Haematol; (43):539-541 1976

Infection of fibroblasts by avian sarcoma viruses (ASV) leads to neoplastic transformation of the host cell, which may be the result of a single viral gene. To study the mechanism of transformation, radioactive DNA (cDNAsarc) complementary to nucleotide sequences that represent most or all of the viral gene(s) responsible for transformation was prepared. The distribution of nucleotide sequences homologous to cDNAsarc among various tumor viruses and among the nucleic acids extracted from normal virus-transformed cells is described. Hybridization experiments were performed between cDNAsarc and a large excess of the viral RNA or DNA to be tested. All the strains of ASV tested contained nucleotide sequences closely related to cDNAsarc. RNA from avian leukosis viruses and temperature-dependent mutants isolated from ASV strains showed no hybridization with cDNAsarc, although they were competent for hybridization with cDNAb77 (DNA complementary to the RNA genome of the Bratislava 77 strain of ASV). No sequences homologous to cDNAsarc were found with RNA from viruses oncogenic in mammals, and negative results were also found for DNA tumor viruses tested. Normal avian DNA contained nucleotide sequences related to cDNAsarc. It appears that cDNAsarc represents a nucleotide sequence that arose and diverged during avian speciation; part or all of the sequences homologous to cDNAsarc in ASV were probably derived from the chicken genome or a species closely related to the chicken. Experiments also supported the idea that the sequences homologous to cDNAsarc in ASV evolved from cellular nucleotide sequences but have been altered considerably in the process.

56. A BIOCHEMICAL AND GENETIC ANALYSIS OF MAMMALIAN RNA-CONTAINING SARCOMA VIRUSES.

Scolnick EM, Goldberg RJ, Park WP
Tumor Viruses Cold Spring Harbor Symposia on Quantitative Biology; Cold Spring Harbor Laboratory (Cold Spring Harbor, NY) (39):885-896 1975

Current studies have shown that all mammalian sarcoma-producing viruses, whether isolated from laboratory experiments or naturally occurring tumors, are deletion mutants of replicating mammalian type C viruses. Nonproducer cells transformed by any of these sarcoma viruses contain RNA homologous to mammalian leukemia viruses, even though the cells contain no known proteins currently coded for by the mammalian leukemia virus. This mammalian leukemia virus information, fibroblast-transforming negative (FT-), is a genetically stable part of the mammalian sarcoma viruses that are fibroblast-transforming (FTI). Another component in the Kirsten and Harvey sarcoma viruses can be identified in addition to this leukemia virus information for the homologous leukemia virus; at least part of the additional information came from rat type C viruses from the animals in which the sarcoma viruses were isolated. This indicates that these two mammalian sarcoma viruses are recombinants between mouse leukemia virus and genetic information in rat

cells and suggests that sarcoma virus formation is analogous to transduction of information in bacteriophage. The Kirsten sarcoma virus seems to have a third component in it separate from either the mouse leukemia virus or rat leukemia virus information. An FTI leukemia virus isolated from mice, the Abelson leukemia virus, is also defective and can be shown to have information homologous to Moloney leukemia virus. In the feline sarcoma virus, feline leukemia information can be detected but no other cat virus information can be. Finally, mutants of Kirsten sarcoma virus temperature-sensitive for the maintenance of transformation have been isolated, and out of 10 such mutants, no complementation has been observed.

57. DELETION MAPPING OF MOLONEY TYPE C VIRUS: POLYPEPTIDE AND NUCLEIC ACID EXPRESSION IN DIFFERENT TRANSFORMING VIRUS ISOLATES.

Parks WP, Howk RS, Anisowicz A, Scolnick EN
Lab. of Tumor Virus Genetics, Natl. Cancer Inst., Bethesda, MD 20014
J Virol; 18(2):491-503 1976

Because each of the genomes responsible for fibroblast transformation (FT) appears to be a recombinant with part of the genome Moloney murine leukemia virus (Mo-MuLV), the Mo-MuLV nucleic acid sequences remaining in each of the FTI isolates were correlated with the Mo-MuLV structural polypeptides synthesized in transformed heterologous nonproducer cells. To measure the Mo-MuLV nucleic acid sequences, viral complementary DNA (cDNA) was fractionated by hybridization to RNA from different nonproducer cells. Because of the differing patterns of polypeptide expression, the transformed nonproducer cells were analyzed for RNA homologous to Mo-MuLV; in correlation with the polypeptide differences, different portions of the Mo-MuLV genome were expressed in these cells. Hybridization showed that a portion of the murine type C genome was present in all the replication-defective murine FTI viruses. These results suggest that the recombination event leading to FTI virus formation occurs at a common region of the murine type C viral genome. A correlation was also found between the final extents of Mo-MuLV RNA expressed and the number of structural polypeptides produced; this suggests that the cDNA probes prepared by cycling against different nonproducer RNA's represent at least part of the sequences coding for p30 and/or the low-molecular-weight proteins. That the patterns of RNA or protein expression never changed in the transformed nonproducer cells indicates that these FTI viruses are deletion mutants and that the RNA and protein expression patterns noted are due to differing deletion of the Mo-MuLV genome and not to transcriptional or translational blocks. Deletion mapping of Mo-MuLV is possible and, by correlating the proteins synthesized with the RNA expressed, one can prepare cDNA probes representing different portions of the Mo-MuLV genome.

58. POLYOMA VIRUS - A STUDY OF WILD-TYPE, MUTANT AND DEFECTIVE DNAs. (PP. 45-52)

Fried M, Griffin BE, Lund E, Roberson DL
Tumor Viruses Cold Spring Harbor Symposia on Quantitative Biology; Cold Spring Harbor Lab. (Cold Spring Harbor, NY): vol. 39, 1975

The restriction enzyme fragments of the polyoma wild-type strain A-2 and the restriction enzyme cleavage patterns of different temperature-sensitive (ts) mutants and purified defective DNA isolates are analyzed. A physical map of polyoma DNA is described. Preliminary experiments indicate that the transforming activity of polyoma DNA is located in the Hemophilus influenzae endonuclease III-55% fragment. The H parainfluenzae endonuclease II cleavage patterns for a

number of ts mutants were tabulated. Various defective molecules, which result from the passage of polyoma virus at high multiplicity, have been cloned in the presence of infectious helper molecules. Defective DNA from these clonal isolates has been purified; it is resistant to *Escherichia coli* restriction enzyme I cleavage and interferes with the production of infectious virus. Further analysis of three defective molecules (D-92, D-74, and D-50) with restriction enzymes allowed the construction of physical maps for the D-92 and D-50 species. The defective molecules may be useful in defining the wild-type genome.

59. LOCALIZATION OF GENE FUNCTIONS IN POLYOMA VIRUS DNA.

Feunteun J, Sompayrac L, Fluck M, Benjamin T
Department of Pathology, Harvard Medical School,
Boston, Massachusetts 02115
Proc Natl Acad Sci USA; 73(11):4169-4173 1976

Polyoma virus mutants of four functionally distinct groups have been mapped by the marker rescue technique using restriction enzyme fragments of wild-type viral DNA. Nontransforming host-range mutants map in the proximal part of the early region of the viral genome. The same DNA fragment that restores a normal host range also restores normal transforming ability to these mutants. ts-25D, a temperature-sensitive (ts)-a class mutant, maps in the distal part of the early region. ts-3 and ts-1260 map in the proximal and distal parts of the late region, respectively. (Author Abstract)

60. THE STRUCTURE AND BIOLOGICAL PROPERTIES OF HERPES SIMPLEX VIRUS DNA. (PP. 657-666)

Wilkie NM, Clements JB, Macnab JC, Subak-Sharpe JH
Tumor Viruses Cold Spring Harbor Symposia on
Quantitative Biology; Cold Spring Harbor Lab. (Cold
Spring Harbor, NY); vol. 39, 1975

Escherichia coli and *Hemophilus influenzae* endonucleases were applied to the study of various herpesvirus DNA's. The distribution and nature of single-strand interruptions after subjecting the DNA to varying degrees of shear stress (by passage through a fine-gauge needle) were determined by electrophoresis. Herpes simplex virus type 1 (HSV-1) broke down to an apparently random assortment of fragments, suggesting the absence of preferred shear sites. The calcium phosphate precipitation technique was extended to baby hamster kidney (BHK) cells and to the DNA's of several other herpesvirus species. Virions produced after infection with DNA from virions of differing genotypes were identical to the original phenotype. Shearing abolished the plaque-producing ability. Some marker genes could be recovered from sheared fragments. DNA (intact or sheared) from temperature-sensitive and permissive HSV types 1 and 2 was used to infect primary rat embryo cell cultures. In most cases, infection with intact DNA led to plaque formation at the appropriate temperatures. With the type 1 mutants ts F, ts G, and ts I and the type 2 mutants ts 1 and ts 3, transformed foci were seen at 38 C. Transformation is clearly independent of the conditional lethal function of these mutants. Since fragments and conditional lethals can transform, it seems unlikely that the whole genome of herpes simplex virus is necessary for successful transformation.

D. Identification and Study of Viral Gene Products

61. DEOXYRIBONUCLEIC ACID POLYMERASE ACTIVITIES IN NORMAL AND LEUKOVIRUS-INFECTED CHICKEN EMBRYO CELLS.

Weissbach A, Bolden A, Muller R, Hanafusa H, Hanafusa T
Roche Inst. Molecular Biol., Nutley, N.J.
J Virol; 10(3):321-327 1972

DNA polymerases were studied in normal chick embryo cells, in chick cells infected with Rous-associated virus (RAV-2), and in chick cells infected with Rous sarcoma virus, type alpha, a noninfectious Rous virus mutant deficient in viral RNA-dependent DNA polymerase (R-DNA polymerase). The R-DNA polymerase of the RAV-2 virion could utilize (dG)12.rC as a template, while the cellular R-DNA polymerase of the chick embryo cell could not copy this template under any circumstances. Chicken cells transformed by RSValpha lacked detectable viral R-DNA polymerase, though cellular R-DNA polymerase was present in normal amounts.

62. REVERSE TRANSCRIPTASE IS CODED FOR BY THE VIRAL RNA.

Verma IM, Hunter E
Salk Inst., Post Office Box 1809, San Diego, CA 92112
Bibl Haematol; (43):457-459 1976

The thermolability of DNA polymerase activities from several temperature-sensitive (ts) mutants of avian and murine RNA tumor viruses was studied to determine whether reverse transcriptase is coded for by the viral RNA. The viruses studied were Rous sarcoma virus Prague strain (PR-C), avian sarcoma virus strain Bratislava 77 (B77-C), ts LA335 PR-C, ts LA337 PR-C, ts LA336 B77-C, Rauscher murine leukemia virus (R-MuLV), and a mutant of R-MuLV, ts 29. Both the DNA polymerase and RNase H activities of all three mutants of avian sarcoma viruses were 5 to 20 times more rapidly heat inactivated than the corresponding wild-type enzymatic activities. Approximately 5 to 10 times less viral DNA was synthesized by three mutants at nonpermissive temperatures than was synthesized by the wild-type virus. The DNA-dependent DNA polymerase and RNase H activities of purified reverse transcriptase from ts 29 were more thermolabile than the corresponding wild-type enzymatic activities. The covariation of the thermolability of DNA polymerase and RNase H activities of ts 29 suggested that, like the avian RNA tumor viruses, the two activities were an integral part of the reverse transcriptase activity from murine leukemia viruses. The inability of the mutant viruses to establish infection and transformation at nonpermissive temperatures was due to a lesion in their virion reverse transcriptase. The data indicate that reverse transcriptase is coded for by the viral RNA and is essential to establish infection by RNA tumor viruses.

63. CHARACTERIZATION OF THE MAJOR VIRAL POLYPEPTIDE IN CELLS TRANSFORMED BY WILD-TYPE AND TEMPERATURE-SENSITIVE MURINE SARCOMA VIRUS.

Spira G, Dreesman GR, Benyeshi-Melnick M, Kit S, Somers KD
Baylor Coll. Med., Houston, Tex.
Intervirology; 4(2):99-109 1974

Extracts of Moloney murine sarcoma virus (M-MSV)-transformed rat cells were analyzed by a radioimmunoassay procedure that detects the 30,000-dalton major virus structural polypeptide (p30). With the appropriate choice of antiserum and competing protein, type-specific p30 antigenic determinants of Moloney leukemia virus were detected in

extracts of producer and nonproducer M-MSV-transformed cells. The specific antigenic determinants were expressed at permissive and restrictive temperatures in cells transformed by a cold-sensitive M-MSV mutant, suggesting that expression of type-specific p30 antigens is not required for maintenance of the transformed phenotype. In parallel experiments, Rauscher leukemia type-specific p30 antigenic determinants were detected in extracts of nonproducer M-MSV-transformed cells superinfected with Rauscher leukemia virus. The intracellular p30 antigen in extracts of wild-type and mutant M-MSV-transformed cells were characterized by Sephadex chromatography and isoelectric focusing. Extracts of M-MSV-transformed cells contained a single peak of p30 antigenic activity with a molecular weight of approximately 30,000 daltons and a isoelectric point of 6.0, suggesting that the polypeptide in cellular extracts was similar in molecular weight and isoelectric point to the corresponding protein isolated from purified Moloney murine sarcoma-leukemia virus.

64. GAG GENE OF MAMMALIAN TYPE-C RNA TUMOUR VIRUSES.

Barbacid M, Stephenson JR, Aaronson SA
Lab. RNA Tumor Viruses, National Cancer Inst.,
Bethesda, MD 20014
Nature (Lond); 262(5569):554-559 1976

Radioimmunoassays were used to study the components and the genetic sequence of the gag gene of a temperature-sensitive Rauscher murine leukemia virus (MuLV) mutant defective in precursor-polypeptide cleavage (mutant ts25). NIH/3T3 mouse cells were infected with ts25 at nonpermissive temperatures, and the various polypeptide precursors and their intermediate cleavage products were studied. Antigenic activities corresponding to four peptides (p30, p15, p12, and p10) migrated as a single precursor (Pr65) with a molecular wt of 65,000-68,000. Various polypeptides containing the individual peptides were present with molecular wt lower than 65,000. The authors analyzed the translation products of various mammalian sarcoma virus-transformed cells in order to sequence the type C viral gag gene. The data strongly suggest that p15 is the amino-terminal protein of the gag gene product and that p10 is the carboxy-terminal protein. The entire gene sequence is thought to be NH₂-p15-p12-p30-p10-COOH. Since the viral RNA genome is the positive strand, the genetic information in the gag gene would be arranged in an identical manner from the 5' to 3' end of the RNA molecule. The authors present the evidence and reasoning that led them to suggest that the type C viral genome contains one protein synthesis initiation site at the 5' end and that the gag gene is close to the end of the RNA molecule.

65. SPONTANEOUS MUTATIONS AFFECTING THE HOST RANGE OF THE B77 STRAIN OF AVIAN SARCOMA VIRUS INVOLVE TYPE-SPECIFIC CHANGES IN THE VIRION ENVELOPE ANTIGEN.

Zarling DA, Mosser AG, Temin HM
Biophysics Laboratory, University of Wisconsin, Madison,
Wisconsin 53706
J Virol; 21(1):105-112 1977

Previously it was shown that the host-range gene of the Bratislava strain of avian sarcoma virus (B77 virus) spontaneously mutates with a very high rate. The wild-type B77 virus, called B77 virus-II, mutates either to virus that efficiently infects duck cells (B77 virus-III) or to virus that does not mutate to the ability to infect duck cells (B77 virus-I) (Zarling and Temin, 1976). No significant differences in either the virion envelope glycoproteins or other major virion proteins were detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. However, pseudotypes of B77 virus-I with proteins of a transformation-defective mutant of B77 virus-III formed foci efficiently in duck cells. An

alteration in the envelope protein of B77 virus-I was demonstrated by experiments in which B77 virus-I was fused into duck cells with UV-irradiated Sendai virus and formed foci. Neutralization experiments further demonstrated that B77 virus host-range mutants have altered type-specific envelope antigens. Thus, the spontaneous mutations in the host-range gene of B77 virus involve changes in the type-specific virion envelope antigen. (Author Abstract)

66. INFECTION OF THYMIDINE KINASE-DEFICIENT BHK CELLS WITH POLYOMA VIRUS.

Littlefield JW, Basilico C
Massachusetts General Hosp., Boston, MA
Nature; 211(5046):250-252 1966

Biochemical variants of baby hamster kidney cells (BHK) deficient in thymidine kinase were selected to investigate the following questions: (a) could infection of such a variant by polyoma virus cause a transient increase in kinase activity and (b) could such infection give rise to an occasional transformed cell that acquires thymidine kinase activity permanently by integration of the viral genome, by transduction, or by derepression or reverse mutation in the host cell genome. Thymidine kinase-deficient cells are unable to phosphorylate 5-bromodeoxyuridine (BUdR); hence they do not incorporate it into their DNA and are protected against the lethal effects of such incorporation. First, clones partially resistant to BUdR were selected from BHK 21/13 cells, and then two markedly BUdR-resistant cell lines were selected from these partially resistant cells by increasing the BUdR concentration in the cultures. The kinase deficiency was reflected in the low rate of incorporation of C14-thymidine into the DNA of growing cells from these lines (B1 and B2). Cell line B1 seemed normally susceptible to polyoma virus transformation, whereas B2 cells were less susceptible. Infection of the kinase-deficient cells with polyoma virus did not appear to cause an increase in kinase activity in comparison with untransformed cells. By inhibiting endogenous thymidine synthesis with aminopterin and providing thymidine in the medium, transformed cells that had permanently acquired thymidine kinase could be selected, if they arose; none were found. (14 Refs)

67. ORIGIN OF THE THYMIDINE KINASE INDUCED BY POLYOMA VIRUS IN PRODUCTIVELY INFECTED CELLS.

Basilico C, Matsuya Y, Green H
New York U. Sch. Med., N. Y.
J Virol; 3(2):140-145 1969

In 3T3 mouse cells, polyoma virus (PV) infection was accompanied by an increase in thymidine kinase (TK) activity. Two lines of 3T3 cells, resistant to 5-bromo-2'-deoxyuridine, were isolated. No increase in TK levels was observed after productive PV infection of these TK-cells, suggesting that TK is not coded for by a PV gene.

68. SIMIAN VIRUS 40 DEOXYRIBONUCLEIC ACID SYNTHESIS: THE VIRAL REPLICON.

Tegtmeyer P
Dept. Pharmacol., Case Western Reserve U., Cleveland, O.
J Virol; 10(4):591-598 1972

Temperature-sensitive (ts) mutants of SV40 (tsA7, tsA28 and tsA30) were used to infect African green monkey kidney cells and DNA production by ts mutants at 41°C was observed. The three ts mutants were deficient in DNA production at this temperature. It appeared that a host cell function could not substitute for the ts restricted virus function. Temperature-shift studies were undertaken to detect variations in kinetic characteristics of the three ts mutants and to determine the effects of temperature changes on their functions. The results indicated that the initiation of replication in the ts viruses ceased at the time of, or shortly after, temperature shifts, and

that mutant replicative intermediate DNA molecules which had already been initiated before temperature shifts continued the replication process to completion. The synthesis of mutant DNA molecules themselves was initiated by a nonmutant gene product in viral complementation studies at 41 C.

69. RELATIONSHIP OF REPLICATION AND TRANSCRIPTION OF SIMIAN VIRUS 40 DNA (MEETING ABSTRACT).

Cowan K, Tegtmeyer P, Anthony DD
Dept. Pharmacol., Case Western Reserve U., Cleveland, Ohio

Proc Natl Acad Sci USA; 70(7):1927-1930 1973

RNA produced by the Simian virus 40 (SV40) mutant tsA30 during lytic infection of kidney cells of African green monkeys was examined by RNA-DNA competition-hybridization. This mutant is temperature-sensitive, in a function (gene A) that regulates synthesis of viral DNA. No detectable difference between mutant RNA synthesized at the permissive temperature (33 C) and wild-type viral RNA was found. During continuous infection with the mutant at the restrictive temperature (41 C) only early viral RNA was produced. When mutant DNA and late RNA synthesis were initiated at the permissive temperature, a shift to the restrictive temperature rapidly terminated synthesis of viral DNA but not that of late viral RNA. Since late RNA synthesis was consistently detected in infection by the mutant 2.5-6.5 hr after a temperature shift to 41 C, the replicative intermediate molecules are apparently not necessary for the continued transcription of late viral genes. The patterns of RNA synthesis in lytic infection, in abortive infection, and in transforming infection are different. It has not been determined whether a common mechanism regulating transcription is operating in each of these states. The data indicate that the function of gene A is required before synthesis of late viral RNA and that after initiation, the production of late RNA continues without further expression of gene A or concomitant viral DNA synthesis.

70. POLYOMA GENE FUNCTION REQUIRED FOR VIRAL DNA SYNTHESIS.

Francke B, Eckhart W
Salk Inst., La Jolla, Ca.
Virology; 55(1):127-135 1973

The ts-a mutant of polyoma virus, and other mutants of the ts-a type, were previously shown to be defective in viral DNA synthesis, T-antigen synthesis and stable transformation at the restrictive temperature of 39 C. The present kinetic study, carried out with mutant-infected BALB/3T3 cells, showed that viral DNA synthesis ceases following a shift from the permissive (32 C) to the restrictive temperature. Inhibition of RNA synthesis by actinomycin D or of protein synthesis by cycloheximide during a shift up in temperature has no effect on the cessation of viral DNA synthesis. Pulse-chase experiments showed that viral DNA molecules already initiated at 32 C could be completed at 39 C and suggested that initiation of new rounds of viral DNA synthesis is blocked at the restrictive temperature. Completion of rounds of viral DNA synthesis at the restrictive temperature occurs at the same rate in mutant-infected cells as in wild-type infected cells. It appeared likely that a viral gene product is required for initiation of rounds of viral DNA replication in lytic infection and also for stable transformation.

71. POLYOMA T ANTIGEN SYNTHESIS BY TEMPERATURE-SENSITIVE MUTANTS OF POLYOMA VIRUS.

Oxman MN, Takemoto KK, Eckhart W
Children's Hosp. Med. Ctr. Boston, Mass
Virology; 49(3):675-682 1972

The production of T antigen detectable by an indirect fluorescent antibody technique was studied in secondary mouse embryo cell cultures infected by one 'late' and four 'early' temperature sensitive mutants of polyoma virus. Plaque assays using infectious viral DNA showed that all four early mutants are defective in viral DNA synthesis at 39 C, whereas the late mutant and wild type viruses are not. All four early mutants were also unable to synthesize T antigen at this higher temperature. However, the fact that inhibition of viral DNA synthesis by cytosine arabinoside or 5-fluorodeoxyuridine did not prevent T antigen synthesis by wild type virus or by the early mutants at the permissive temperature suggested that the failure of the early mutants to produce T antigen at 39 C was not due to defective DNA replication per se. Rather, the inability to produce T antigen at the non-permissive temperature may reflect an alteration in either the gene which codes for T antigen, or in a gene whose expression is necessary for both T antigen production and for viral DNA replication.

72. THERMOLABILE T (TUMOR) ANTIGEN FROM CELLS TRANSFORMED BY A TEMPERATURE-SENSITIVE MUTANT OF SIMIAN VIRUS 40 (MEETING ABSTRACT).

Tenen DG, Baygell P, Livingston DM
Sidney Farber Cancer Center, Boston, Mass. 02115
Proc Natl Acad Sci USA; 72(11):4351-4355 1975

Partially purified tumor (T) antigen from a strain of Chinese hamster lung cells transformed by wild-type Simian virus 40 (SV40) and either of two temperature-sensitive SV40 mutants was studied as a DNA-binding protein. The DNA binding activity present in the T-antigen-containing fractions was inhibited by purified hamster anti-T IgG but not by equivalent amounts of nonimmune hamster IgG. T from either wild-type- or tsC219-transformed cells was relatively stable during heating at 44 C compared to T antigen prepared from tsA239-transformed cells. The latter antigen lost half of its activity in one hour at 44 C, and 30% in two hours at 30 C. Antigen from wild-type cells lost less than 10% of its activity during these treatments. These results strongly suggest that T antigen is a product of the SV40 A gene.

73. POLYOMA VIRUS T ANTIGEN. I. SYNTHESIS OF MODIFIED HEAT-LABILE T ANTIGEN IN CELLS TRANSFORMED WITH THE TS-A MUTANT.

Paulin D, Cuzin F
Dep. Mol. Biol., Inst. Pasteur, Paris, France
J Virol; 15(2):393-397 1975

The synthesis of T antigen in mouse 3T3 cells transformed either by wild type (PY-3T3 PY-6) or by a temperature-sensitive mutant of polyoma (ts-a-3T3) virus was investigated to determine if T-antigen is the product of a viral gene. Cells were grown on cover slips at various temperatures, fixed, and stained by the indirect immunofluorescence technique, using polyoma anti-T hamster serum and fluorescein-coupled anti-hamster immunoglobulin (goat) antiserum. In Py-6 cells grown at 39 C or shifted for various periods of time up to 48 hr at 31 C, a typical immunofluorescence staining was observed in more than 80% of the cells. Correspondingly high antigen concentrations were measured in extracts by complement fixation assays. In mouse cells transformed with ts-a-3T3, only low amounts (1 to 1000 cells) of the virus specific T antigen were synthesized at high temperature (39 C). After a shift-down to the permissive temperature (31 C), these cells

exhibited the same level of T-antigen production as wild type polyoma transformants. The T-antigen produced by ts-transformed cells was inactivated at 39 C in vitro at a faster rate than that produced by wild type transformed cells. Loss of T-antigenic activity in extracts at 39 C, correlated with the ts genotype of the virus and the absence of detectable T antigen in 95% of cells growing at this temperature, strongly suggests that the product of the viral gene carries the T-antigenic site.

74. CHARACTERIZATION OF T ANTIGEN IN CELLS INFECTED WITH A TEMPERATURE-SENSITIVE MUTANT OF SIMIAN VIRUS 40.

Kuchino T, Yamahuchi N

Inst. Med. Sci., Univ. Tokyo, Tokyo, Japan

J Virol; 15(6):1302-1307 1975

T antigen induced in African green monkey kidney cells by a temperature-sensitive mutant of simian virus 40 (ts 900), defective in a function required for cell transformation was characterized. The number of T antigen-positive cells estimated by an immunofluorescent technique was almost equal at permissive (32.5 C) and restrictive (38.5 C) temperatures, (the ratios of positive nuclei at 38.5 C to those at 32.5 C were 1.4 and 1.3 for the heat-labile and wild type viruses, respectively), but was slightly reduced when the infected cells were incubated at a higher temperature (40.5) (ratios of 2.2 and 0.66 respectively). However, a complement fixation test indicated that the amount of T antigen induced by the mutant was not significantly different from that induced by wild-type virus at 40.5 C. These results suggest that the T antigen-inducing ability of the mutant is not defective. Two distinct molecular species of T antigen were induced by the mutant at the permissive temperature, whereas only one form was observed at the restrictive temperature. The larger molecular form (14 to 15S) induced by the mutant at the permissive temperature was more heat labile than that induced by wild-type virus, suggesting that the mutated gene product may be T antigen or another component of the molecular form of the antigen.

75. REGULATION OF TUMOR ANTIGEN SYNTHESIS BY SIMIAN VIRUS 40 GENE A.

Tegtmeyer P, Schwartz M, Collins JK, Rundell K

Dept. Microbiology, State Univ. New York Stony Brook, Stony Brook, N.Y. 11794

J Virol; 16(1):168-178 1975

The effect of the simian virus 40 gene A function on proteins synthesized during productive and transforming infections is examined. Under restrictive conditions, temperature-sensitive A mutants induce the overproduction of a 100,000-dalton protein both in productively infected monkey cells and in transformed rabbit cells. Immuno-precipitation of the induced protein with antisera, prepared against simian virus 40-induced tumors in hamsters, was used to identify the induced protein as tumor antigen. The same protein could be precipitated from extracts of cells infected by wild-type virus but not from uninfected cells. Furthermore, the mutant-induced protein was more rapidly degraded in vivo and was less tightly bound to intranuclear components than the protein induced by wild-type virus. The presence of the same virus-induced protein in infected cells from different species and the altered behavior of that protein in mutant infection strongly suggest that the protein is virus coded. Because the protein is large enough to account for the entire coding capacity in the early region of the Simian virus 40 genome, the 100,000-dalton protein may well be the primary product of the only early gene identified by complementation studies, the A gene. If the 100,000-dalton protein that is overproduced in mutant infection is the A protein and the only early protein, then functional wild-type A protein must regulate its own synthesis in both productive and transforming infections.

76. ALTERED PATTERNS OF PROTEIN SYNTHESIS IN INFECTION BY SV40 MUTANTS. (PP. 9-15)

Tegtmeyer P

Tumor Viruses Cold Spring Harbor Symposia on

Quantitative Biology; Cold Spring Harbor Lab. (Cold Spring Harbor, N.Y.): vol. 39, 1975

In vivo protein synthesis in infection by early and late temperature-sensitive mutants of SV40 was studied; infection by the early A mutants and the late mutant B11 was emphasized. Protein synthesis patterns in cells infected by wild-type and mutant virus were compared by short periods of ³⁵S-methionine labeling. Protein synthesis patterns in uninfected cells varied in the relative quantities of individual proteins synthesized (depending upon incubation temperature), length of the radiolabeling period, and cell growth state. Except for inducing the synthesis of virion proteins (VP) and cellular histones, infection by wild-type virus resulted in only minor changes from the pattern in uninfected cells. Both the intracellular distribution and stability of VP1 are altered in infection by B11, suggesting that the major capsid protein may be the altered protein of the mutant. The most striking alteration in B11 infection was increased synthesis of specific nonvirion proteins, apparently of cellular origin. The most significant finding in A mutant infection at 41 C was the induction of a high molecular weight (100,000 daltons) protein with a rapid intracellular turnover that was identifiable after short periods of radio-labeling. Cellular extracts (known to contain induced proteins) were exposed to hamster anti-T serum to immunoprecipitate proteins possibly related to early viral function and to transformation. None of the B11-induced proteins was precipitated. The A58 100,000-dalton protein was detected in cells infected with wild-type virus or B11 but not in uninfected cells. It is suggested that this protein may be the only early viral protein; it may regulate its own synthesis since it is overproduced in A mutant infection.

77. CHARACTERIZATION OF POLYOMA VIRUS T ANTIGEN.

Ito Y, Spurr N, Dulbecco R

Department of Cell Regulation, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX England
Proc Natl Acad Sci USA; 74(3):1259-1263 1977

High-titer antiserum raised in rats against the tumor (T) antigen of polyoma virus was used to purify the T antigen by the Staphylococcus protein A antibody adsorbent technique. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis allowed the identification of a protein with an apparent molecular weight of 100,000-108,000 as a major component induced in lytically infected mouse cells. In cells infected by ts A mutants this component was temperature sensitive. Several minor components were also observed. In pulse and chase experiments there was a slight decrease in electrophoretic mobility of T antigen during the chase period at the permissive temperature, suggesting that the T antigen is a modified protein. In two lines of transformed cells, the amount of T antigen seemed to be considerably less than in lytically infected cells, but the size of the antigen appeared to be equal. (26 Refs) (Author Abstract)

78. IDENTIFICATION OF SIMIAN VIRUS 40 TUMOR AND U ANTIGENS.

Robb JA

Department of Pathology, University of California at San Diego, La Jolla, Calif. 92093

Proc Natl Acad Sci USA; 74(2):447-451 1977

The synthesis and identity of the tumor and U antigens of simian virus 40 (SV40) have been examined during productive infection in monkey cells, abortive infection in mouse cells, and in SV40-transformed mouse cells by using sodium dodecyl

sulfate/polyacrylamide slab gel electrophoresis to analyze (³⁵S)methionine-labeled radioimmune precipitates. The following observations were made: (i) the tumor and U antigenic sites are on the same 94,000, 89,000, and 84,000 molecular weight species detected during productive infection; a 94,000 species made during abortive infection; and a 94,000 species found in transformed cells. (ii) The 94,000 species is relatively unstable compared to the relatively stable 89,000 and 84,000 species produced during productive infection. (iii) The stable 89,000 and 84,000 molecular weight species are differentially extracted from productively infected cells, which suggests an intracellular compartmentation and/or different affinities of these species for cellular substrates. (iv) The 94,000 species synthesized during abortive infection is more stable than the comparable 94,000 species synthesized in transformed cells. (v) Three tsA group mutants overproduce several unstable species of tumor antigen at restrictive temperature. (Author Abstract)

79. BIOLOGY OF SV40 TRANSPLANTATION ANTIGEN (TRAG). I. DEMONSTRATION OF SV40 TRAG ON GLUTARALDEHYDE-FIXED SV40-INFECTED AFRICAN GREEN MONKEY KIDNEY CELLS.

Tevethia MJ, Tevethia SS

Tufts Univ. Sch. Medicine, Boston, MA 02111

Virology; 69(2):474-489 1976

The synthesis of papovavirus Simian virus 40 (SV40) transplantation antigen (TrAg) in SV40-infected permissive African green monkey kidney cells (TC7) was demonstrated with the *in vivo* transplantation-rejection test. Adult BALB/c mice immunized with SV40-infected cells, which had been fixed with 0.25% glutaraldehyde for 5 min at room temperature, rejected a challenge of syngeneic virus-free SV40-transformed mouse cells (VLM). Fixed uninfected TC-7 cells failed to induce transplantation immunity against VLM cells in mice. The induction of SV40 tumor immunity in mice after immunization with glutaraldehyde-fixed infected TC-7 cells was mediated by the TrAg synthesized in permissive monkey cells as a result of virus infection, rather than by the residual infectious virus in the glutaraldehyde-fixed infected cells. The treatment of infectious SV40 with 0.25% glutaraldehyde drastically reduced both viral infectivity and tumor immunity-inducing ability of the virus. Glutaraldehyde-inactivated virus also failed to induce SV40 T antigen in monkey cells, thus ruling out the possibility that the inactivated cell-associated virus was responsible for the induction of tumor immunity *in vivo*. The amount of infectious SV40 required to induce detectable tumor immunity was 2×10^5 plaque-forming units (PFU) of virus administered to adult mice, and was far greater than the amount of virus (1×10^4 PFU) that remained associated with SV40-infected monkey cells after fixation of cells with glutaraldehyde. The synthesis of SV40 TrAg in SV40-infected TC-7 cells was inhibited by 5 µg/ml of actinomycin D, but not by 15 µg/ml of cytosine arabinoside, indicating that transcription in the infected cells, but not viral DNA synthesis, is required for the expression of TrAg. Using 0.25% glutaraldehyde to fix infected cells, it should be possible to test available temperature sensitive mutants of SV40 for their ability to synthesize TrAg at the nonpermissive temperature, since the glutaraldehyde fixation reduces infectivity of intracellular and extracellular virus, stops the infectious cycle at a particular stage, and preserves immunogenicity of TrAg.

80. TUMOR-SPECIFIC TRANSPLANTATION ANTIGEN IS EXPRESSED DURING SV40 LYTIC INFECTION WITH WILD-TYPE AND TSA MUTANT VIRUSES.

Anderson JL, Martin RG, Chang C, Mora PT

Lab. Molecular Biology, Natl. Inst. Arthritis, Metabolism, and Digestive Diseases, NIH, Bethesda, MD 20014

Virology; 76(1):254-262 1977

The expression of simian virus 40 (SV40) tumor-specific transplantation antigen (TSTA) in SV40-infected monkey cells was assayed at permissive and restrictive temperatures using detergent extracts of cell membranes and cytosol to immunize Balb/c female mice prior to an SV40 ascites-tumor challenge. TSTA was expressed during infection of CV-1 monkey kidney cells by wild-type SV40 and by a temperature sensitive (ts) A mutant. The tumor-rejecting capacity induced by the detergent extracts of cell membranes was specific: (1) a non-SV40 tumor line (a methylcholanthrene-induced tumor) was not rejected, and (2) detergent extracts from SV40 but not polyoma-transformed cells were protective. TSTA was induced in wild-type infection and in tsA mutant infection at restrictive and permissive temperatures. A kinetic study demonstrated that TSTA emerges early in the lytic cycle; it is present at 14 but not at 6 hr after infection and then increases several times between 14-26 hr. This parallels the appearance of tumor antigen, as assayed by complement fixation. TSTA, a protein of at least 50,000 daltons, might be either a product of the host cell induced by SV40 or a product of the A gene modified in a distinct fashion from TA but possessing at least partial amino acid homology with TA. (34 Refs)

81. ADENOVIRUS-INFECTED, CELL-SPECIFIC, DNA-BINDING PROTEINS. (PP. 559-566)

Levine AJ, Van Der Vliet PC, Rosenwirth B, Rabek J, Frenkel G, Ensinger M

Tumor Viruses Cold Spring Harbor Symposia on Quantitative Biology; Cold Spring Harbor Lab. (Cold Spring Harbor, NY): vol. 39, 1975

Cell-specific DNA-binding proteins were isolated from adenovirus-infected cells and characterized. African green monkey kidney (AGMK) cells were mock-infected or infected with type 5 adenovirus (Ad5) and labeled with ¹⁴C-leucine or ³H-leucine. Two infected, cell-specific, single-stranded DNA-binding proteins were produced with molecular weights of 72,000 and 48,000 daltons. Similar proteins were found in Ad2- and Ad12-infected AGMK cells. The purified DNA-binding proteins bound preferentially and cooperatively to any single-stranded DNA tested, but no binding to double-stranded DNA or 23S Escherichia coli RNA was detected. Approximately 1 to 10×10^6 molecules were synthesized after infection of the AGMK cells, suggesting a stoichiometric rather than a catalytic function for these proteins. Extracts of Ad5 ts 125-infected AGMK cells prepared at the nonpermissive temperature did not contain detectable adenovirus DNA-binding proteins, but these proteins were present in extracts prepared at the permissive temperature. The amount of DNA-binding proteins consistently decreased when Ad5 ts 125-infected AGMK cells were shifted from the permissive to the nonpermissive temperature. The proteins produced at permissive temperatures appeared to be more thermolabile for binding to single-stranded DNA than wild-type proteins. It is possible that the 48,000-dalton protein is a proteolytic breakdown product of the 72,000-dalton protein. SV40 infection of AGMK cells was able to overcome the Ad5 ts 125 block to adenovirus DNA synthesis. SV40 ts B-8 and SV40 ts B-11 mutants, but not SV40 ts A-30, were also able to complement Ad5 ts 125 in AGMK cells. The data suggest that the adenovirus-induced DNA-binding proteins are required for adenovirus DNA replication. Common functional aspects

of adenovirus and SV40 cellular transformation are also suggested.

II. VIRAL MUTATIONS AFFECTING TRANSFORMING ABILITY

A. Mutations of DNA Tumor Viruses Affecting Transforming Ability

82. CELL-TRANSFORMING ABILITY OF A TEMPERATURE-SENSITIVE MUTANT OF POLYOMA VIRUS.

Fried M

Div. Biology, California Inst. Technology, Pasadena, CA
Proc Natl Acad Sci USA; 53(3):486-491 1965

A mutant (TS-a) of polyoma virus originally isolated by its temperature sensitivity in the cytotoxic virus cell interaction was investigated to determine its ability to transform BHK/C13 hamster cells in vitro. Hamster cells were exposed to five concentrations of TS-a (1000, 100, 10, 1, and 0 plaque-forming units (PFU)/cell) and plated in 4 x 15 replicates from each treatment; the replicates were incubated 15 at a time under four temperature regimes: high temperature (16-18 days at 38.5 C), shift-up (4 days at 31.5 C, then 16-18 days at 38.5 C), shift-down (2 days at 38.5 C, then unspecified days at 31.5 C), and low temperature (32-36 days at 31.5 C). Both the high temperature and shift-down incubation procedures virtually abolished transformation in comparison with the low temperature procedure; but the shift-up procedure yielded essentially the same number of transformed colonies as did the low temperature incubation. These results indicate that the temperature-sensitive gene product is involved in the initiation but not in the maintenance of the transformed state. TS-a was then compared with its parental wild-type, PY, for the ability to carry out transformation in vivo, using 5-day old hamsters. Animals were inoculated sc or ip with either 9×10^4 PFU of TS-a or 2.7×10^4 PFU of PY and observed for 70 days. All the animals inoculated with PY died from the rupture of angiomatous tumors of the liver or lungs 12-40 days after inoculation; most also had macroscopic tumors of the internal organs, chiefly the heart and kidneys; subcutaneous tumors occurred only in the animals that survived the longest. Conversely, TS-a inoculated animals had chiefly subcutaneous tumors; the few internal tumors were all either free or loosely connected to the internal body wall except for one tumor of the heart. These results indicate an inhibition of TS-a mediated tumor formation, with restriction of the tumors chiefly to the lower temperature sc site, and confirm the conclusions of the in vitro study. (11 Refs)

83. 'RESCUED' SV40: INCREASED TRANSFORMING EFFICIENCY IN MOUSE AND HUMAN CELLS (MEETING ABSTRACT).

Todaro GJ, Takemoto KK
NCI, Bethesda, Md.

Proc Natl Acad Sci USA; 62(4):1031-1037 1969

After recovery from transformed mouse (3T3 and BALB/3T3) and human tissue cells (L.S and B.L) the small plaque size mutant of SV40 showed the greatest increase in transforming ability, the minute plaque size less and the large plaque size little or none. Neither the minute nor the small plaque viruses could act as a helper for the large. The recovered virus in each case demonstrated the same host range, plaque type, temperature sensitivity and ability to induce cellular DNA synthesis as the parental virus. The production of SV40 T antigen paralleled the transforming ability in both the parental and the recovered viruses. In one viral strain (SV-S-68) increased transforming ability was undiminished even after

two passages through monkey kidney. The mechanism of change in transforming properties was attributed to either a selection process or a host-induced modification.

84. HOST RANGE MUTANTS OF POLYOMA VIRUS (MEETING ABSTRACT).

Benjamin TL

Publ Hlth. Res. Inst., New York, N.Y.

Proc Natl Acad Sci USA; 67(1):394-399 1970

The 3T3 line of Swiss mouse embryo fibroblast was used as the normal line from which a line of polyoma-transformed virus-free cells fully susceptible to lytic infection by polyoma virus was isolated. This line was used to select virus mutants which had lost most or all of their ability to grow in the untransformed parental line while retaining the ability to grow in the transformed derivative; the mutagens used were hydroxylamine (incubation of virus with 0.4M soln at pH 7.0 at 45 degrees for 90 min) and N-methyl-4'-nitro-N-nitrosoguanidine (314 microg/ml for 33 hr starting at the 17th hr of infection). Four virus mutants with the desired properties were isolated (NG-18, NG-23, HA-33 and NG59). These virus mutants were also defective in their ability to transform cells of rat or hamster origin. The DNA extracted from the mutants had the same host range as the whole virus, indicating that the mutants were blocked at some intracellular step which is necessary both for transformation in rat or hamster cells, and for the completion of virus development in mouse cells.

85. ABSENCE OF A CELL MEMBRANE ALTERATION FUNCTION IN NON-TRANSFORMING MUTANTS OF POLYOMA VIRUS (MEETING ABSTRACT).

Benjamin TL, Burger MM

Publ. Hlth. Res. Inst. New York, N.Y.

Proc Natl Acad Sci USA; 67(2):929-934 1970

The ability of polyoma virus infection to expose an agglutinin-binding site on the membrane of cells was investigated. Normal mouse cells cultured with wheat germ agglutinin were infected with polyoma virus (5-10 plaque-forming units/cell), and the ensuing agglutination was observed. Although oncogenic viruses expose the agglutination site on these cell membranes, non-transforming polyoma virus mutants do not (infection of normal mouse cells with these mutants produced 0% agglutination). Transforming wild-type polyoma virus infection, however, did expose the agglutination-site, producing 10-15% agglutination. Inhibitors of DNA synthesis, such as thymidine and 5-fluorodeoxyuridine-5'-P, blocked the exposure of the agglutinin-binding site by wild type polyoma virus, but this blockage was reversed when conditions allowing DNA synthesis were restored. The findings suggest that the agglutinable state of the cell membrane is important in the loss of regulation of cell growth associated with carcinogenesis.

86. STUDIES ON NON-TRANSFORMING HOST RANGE MUTANTS OF POLYOMA VIRUS. (PP. 141-147)

Benjamin TL, Norkin L, Goldman E

Tumor Virus-Host Cell Interaction; Edited by A. Kolber.
(New York: Plenum Press): 1975

The isolation and partial characterization of two kinds of conditional lethal mutants of polyoma virus are reviewed, and experiments on the ability of the nontransforming host range mutant, NG-18, to induce abortive transformation in a continuous line of rat embryo cells are reported. Mutants of the first or initiation class can carry out abortive transformation, stimulate cellular DNA synthesis, and cause agglutinability at the nonpermissive temperature. Mutants of the second or maintenance class uniformly fail to induce agglutinability but show variable responses in inducing cellular DNA synthesis. In experiments with NG-18, normal rat embryo cells were arrested in G1, infected by wild-type polyoma virus or NG-18, and incubated for 48 hr in serum-free medium

containing 2 $\mu\text{Ci}/\text{ml}$ ^3H -thymidine. The number of cells synthesizing DNA was determined by autoradiography. The wild-type virus was 10 times more effective than NG-18 in inducing cellular DNA synthesis and was able to induce abortive transformation, while NG-18 was not. Both were equally effective in inducing the polyoma-specific tumor antigen in serum-starved rat cells. The inability of NG-18 to cause abortive transformation was related to failure to induce cellular DNA synthesis and to bring about the cell surface change from the nonagglutinable to the agglutinable state, which had been shown to depend on cellular DNA synthesis. The relationship between agglutinability and permissivity was tested directly by isolating phenotypic revertants of permissive polyoma-transformed 3T3 cells that had regained normal growth properties. In all three revertants tested for agglutinability by concanavalin A and permissivity for wild-type and NG-18 viruses, the membrane was restored to the agglutinable level characteristic of normal 3T3 cells, while the cells remained largely permissive. It is concluded that both the agglutinable state and the permissive function are expressions of cellular genetic information capable of being triggered by different transforming viruses and that several steps must be involved between the expression of cell-associated viral genes and the cell surface change.

87. ANALYSIS OF HOST RANGE OF NONTRANSFORMING POLYOMA VIRUS MUTANTS.

Goldman E, Benjamin TL

Harvard Medical Sch., Boston, Mass. 02115

Virology; 66(2):372-384 1975

The nontransforming polyoma virus mutant, NG-18 was shown to grow in a variety of other cell types besides polyoma-transformed cells. Among cells found to be substantially permissive for the growth of NG-18 are: phenotypic revertants of polyoma-transformed 3T3 cells, C-type RNA virus transformed and/or producing 3T3 cells, 3T3 cells that become transformed upon infection with leukemia virus, primary baby mouse kidney epithelial cells, and primary mouse embryo fibroblasts. Among cells found to be poor hosts for the growth of NG-18 are: several 3T3 cell lines, SV40-transformed 3T3 cells, spontaneously transformed 3T3 cells, radiation- and chemical carcinogen-transformed 3T3 cells, and late passage mouse embryo fibroblasts. Similar results were obtained using three other independently-isolated host range mutants of the same type as NG-18. The permissive state thus does not require a cell-associated polyoma genome as the basis for complementation of growth for this class of host range mutant. In addition, the results demonstrate that cells can be permissive for the growth of such nontransforming virus mutants without themselves possessing properties commonly associated with transformation such as: loss of density-dependent inhibition of growth, low serum requirement for growth, loss of anchorage dependence of growth, and lectin agglutinability. These findings are discussed in reference to possible mechanisms of action of the NG-18 gene in productive infection and transformation.

88. ONCOGENICITY OF WILD TYPE AND MUTANT STRAINS OF POLYOMA.

Siegler R, Benjamin T

Drew Postgrad. Med. School, Los Angeles, Calif. 90059

Proc Am Assoc Cancer Res; 16:99 1975

This study was designed to compare the oncogenicity of wild type and mutant strains of polyoma virus in new-born hamsters. Polyoma mutant NG-18 was chosen because it induces neither transformation in vitro nor cell surface alterations detected by lectin. Neonatal hamsters were injected IP with various doses of virus. Of 24 animals receiving as little as 5×10^4 pfu (plaque-forming units) of wild type virus, all 24

had tumor within 28 days; in contrast, none of 25 animals injected with as much as 2×10^6 pfu of NG-18 developed tumor in the same period of time. Although the animals receiving NG-18 failed to develop tumors, their sera were positive for antibody against the polyoma T (tumor) antigen, indicating that the initial stages of infection had occurred. Wild type virus tumors consisted of proliferating mesenchymal cells which developed extensive perinuclear granular reticulum and elaborated collagen fibrils. These fibroblasts often first arose in the lamina propria of the dorsum of the tongue. Their growth into the stroma of nearby salivary glands produced characteristic salivary gland tumors. Some animals survived past 30 days, after which regressive changes occurred in the tumors. This study indicates that the non-transforming behavior of the mutant virus in tissue culture appears to correlate with a lack of oncogenicity in the animal. (Author Abstract)

89. DEFICIENCY IN HISTONE ACETYLATION IN NONTRANSFORMING HOST RANGE MUTANTS OF POLYOMA VIRUS (MEETING ABSTRACT).

Schaffhausen BS, Benjamin TL

Harvard Medical Sch., Boston, MA 02115

Proc Natl Acad Sci USA; 73(4):1092-1096 1976

The histone acetylation of wild-type polyoma and simian virus 40 particles, and of nontransforming host range mutants of polyoma virus, was studied by polyacrylamide gel electrophoresis. Histones H3 and H4, derived from transforming wild-type polyoma and simian virus 40 particles were found to show extensive acetylation compared to the histones of the host cells.

90. GENETIC ANALYSIS OF SIMIAN VIRUS 40. IV. INHIBITED TRANSFORMATION OF BALB/3T3 CELLS BY A TEMPERATURE-SENSITIVE EARLY MUTANT.

Robb JA, Smith HS, Scher CD

Natl. Cancer Inst., Bethesda, Md.

J Virol; 9(6):969-972 1972

BALB/3T3 clone A31 mouse cells were absorbed with virions of the temperature-sensitive ts 101 mutant of SV40 (101 virus) at 32 and 38.5 C (permissive and restrictive temperatures, resp). Transformation of cells and synthesis of SV40 tumor (T) antigen were observed. There was no difference between normal wild-type and 101 SV40 virion absorption on BALB/3T3 at 33 or 38.5C. The ability of 101 virions to initiate T antigen synthesis in abortively and stably transformed cells was inhibited 8-20 times at 33 C and 400-500 times at 38.5 C. T antigen synthesis after 101 virion infection was temperature-sensitive. Both abortive and stable transformation of 3T3 cells by 101 virions were inhibited at both 33 and 38.5 C, but neither transformation was temperature sensitive. Two clones of 101-transformed cells from a 33 C stable transformation assay released rescuable virus at 33, 38 and 40 C. All rescued viruses were similar to the original 101 mutant in temperature sensitivity.

91. A TEMPERATURE-SENSITIVE MUTANT OF SIMIAN VIRUS 40 AFFECTING TRANSFORMING ABILITY.

Kimura G, Dulbecco R

Salk Inst., San Diego, Calif.

Virology; 52(2):529-534 1973

In productive infection of monkey BSC-1 cells, ts 640, a temperature-sensitive (ts) mutant of simian virus 40 (SV40), was defective in its ability to synthesize infectious viral DNA and infectious viral particles at the restrictive temperature of 40 degrees, but not at the permissive temperature of 33 degrees. Growth of the mutant after infection with viral DNA was also temperature-sensitive, indicating that the block to

multiplication at 40 degrees is not due to failure in adsorption, penetration or uncoating. Results of mixed infections with other ts mutants showed that the gene identified in this mutant is different from those two cistrons which had been suggested to specify virion proteins. In nonproductive infection of rat 3Y1 cells, ts 640 was temperature-sensitive in its transforming ability at 40 degrees but not at 33 degrees, providing evidence that cell transformation by SV40 is under the control of a viral gene function. The transformed cells obtained at 33 degrees appeared not to revert to normal when shifted to 40 degrees, as judged by their colonial morphology under the standard culture conditions, indicating that the viral gene function under study may be required for the initiation but not for the maintenance of transformation.

92. TEMPERATURE-SENSITIVE MUTANTS OF SIMIAN VIRUS 40 SELECTED BY TRANSFORMING ABILITY.

Yamaguchi N, Kuchino T
Inst. Med. Sci., Univ. Tokyo, Japan
J Virol; 15(6):1297-1301 1975

Eight temperature-sensitive (TS) mutants of Simian virus 40 (SV40) were isolated by UV irradiation or hydroxylamine treatment of wild-type (WT) parental SV40 virus propagated in cultures of African green monkey (AGMK) cells or GC7 cells, a clonal AGMK cell line. The eight mutants were able to transform nonpermissive rat cells at 32.5 C but not at 38.5 C, and were also temperature sensitive for replication in permissive AGMK or GC7 cells. In rat cells, WT virus and a control ts mutant with unimpaired transforming ability formed 1.2- to 2.2-fold more foci at 38.5 C than at 32.5 C, whereas the eight transformation defective mutants formed 9- to 125-fold fewer foci at 38.5 than at 32.5 C. Although these results indicated that a viral function required for formation of dense foci in rat cells is also necessary for SV40 replication in monkey cells, the temperature sensitivity of the ts gene function was expressed differently in transformation and viral replication. For example, the efficiency of plaque formation of two ts mutants in rat cells and permissive GC7 cells was equal at both 38.5 and 32.5 C, but the efficiency of transformation was 30-fold higher at 32.5 than at 38.5 C. Even in this case, the same ts gene product may be required both for transformation and for viral replication since the plaque-forming capacity of one of these two mutants was 50% less than that of WT virus at 38.5 C, and the mutant produced a 1000-fold lower virus yield at 40.5 than at 35 C at a low multiplicity of infection. Five of the ts mutants were classified into one complementation group that may correspond to Tegtmeyer's group A. No mutant incapable of transforming rat cells at either temperature was isolated.

93. BIOLOGICAL ACTIVITIES OF DELETION MUTANTS OF SIMIAN VIRUS 40.

Scott WA, Brockman WW, Nathans D
Dept. Biochemistry, Univ. Miami, Sch. Medicine, Miami, FL 33152
Virology; 75(2):319-334 1976

Mutants of simian virus 40 (SV40) with large deletions in the early or late regions of the genome were tested for biological activity. Two mutants had deletions spanning the two known late genes of SV40 (B/C and D) and had the entire early region intact. Two other mutants had deletions that eliminated 60%-70% of the early region and had the entire late region uninterrupted. The mutants lacking portions of both late genes were able to induce T antigen in infected cells, replicate their DNA in the absence of helper virus, stimulate thymidine incorporation into cellular DNA, and transform mouse and hamster cells. Cells transformed by these late deletion mutants contained the mutant genome, as demonstrated by a fusion-complementation rescue procedure. The deletion mutants lacking substantial portions of the early

genomic region lacked all of these activities. Both early and late deletion mutants, however, interfered with SV40 DNA replication. The finding that the early genome segment of SV40 DNA is sufficient for viral DNA replication indicates that this segment of DNA will be useful as a vector for constructing self-replicating plasmids containing inserted DNA segments. (39 Refs)

94. CHARACTERIZATION OF HOST-RANGE AND TEMPERATURE-SENSITIVE MUTANTS OF ADENOVIRUS TYPE 5 WITH PARTICULAR REGARD TO TRANSFORMATION OF A HAMSTER EMBRYO CELL LINE (NIL).

Minekawa Y, Ishibashi M, Yasue H, Takahashi M
Res. Inst. Microbial Diseases, Osaka Univ., Suita, Osaka, Japan
Virology; 71(1):97-110 1976

Seventeen conditional lethal mutants of (7 host range and 10 temperature sensitive) of adenovirus type 5 (Ad5) were classified by the complementation test and characterized physiologically in viral DNA synthesis, induction of cell DNA synthesis (in hamster kidney cells), capsid polypeptide production, and transformation of Nil cells (a hamster embryo cell line) under restrictive conditions. Seven host-range (hr) mutants were divided into six groups by the complementation test and into three classes by phenotypic characterization. Mutants assigned to Class III (complementation groups D, E, F) were positive in viral DNA synthesis and capsid polypeptides (hexone, pentone base, fiber) production, and showed some degree of leakiness. Class II mutants (complementation groups B, C) were positive in viral DNA synthesis, with a small amount of capsid polypeptide production. Class I mutant (complementation group A) was an early mutant defective in viral DNA synthesis but positive in induction of host DNA synthesis. Transformation of Nil cells was observed with Classes I and II mutants and not with Class III mutants. Ten temperature-sensitive (ts) mutants were divided into seven complementation groups and into five classes by the available phenotypic criteria. Class V mutant (complementation group G) was positive in viral DNA synthesis and capsid polypeptide production with extreme leakiness. Class IV mutants (complementation groups E, F) were positive in viral DNA synthesis and capsid polypeptide production. Class III mutants (complementation groups C, D) were similar to Class IV except for reduced hexone production. Class II mutants (complementation group B) were early mutants defective in viral DNA synthesis but positive in induction of host-DNA synthesis. Class I mutants (complementation group A) were similar to Class II but with a reduced degree of induction of host DNA synthesis. Transformation of Nil cells was observed with Classes II, III, and IV mutants but not with I and V mutants. Thus, the phenotypic characterization of hr and ts mutants by infection of hamster cells showed a good correlation between complementation grouping and the defective function.

B. Mutations of RNA Tumor Viruses Affecting Transforming Ability

95. NONINFECTIOUS RSV DEFICIENT IN DNA POLYMERASE.

Hanafusa H, Hanafusa T
Dept. Viral Oncology, The Public Health Res. Inst., City of New York Inc., New York, NY 10016
Virology; 43(1):313-316 1971

About 10% of the cells transformed by the alpha type Bryan strain of Rous sarcoma virus (RSV) produce structurally intact but noninfectious particles, RSV-alpha(O), as opposed to the remaining 90% which produce infectious particles, RSV-beta(O). A number of alpha type and beta type

RSV strains were assayed for the presence of DNA polymerase, using Rous associated virus (RAV-2) as the standard. In the range of 5-50 microg of RAV-2 protein, the incorporation of tritiated DNA precursor was proportional to the amount of the virus, with the exception of RSV-alpha(O) variants. Their DNA polymerase activity was not above background. Even though the template activity of the RNA of RSV-alpha(O) has not been investigated, the lack of precursor incorporation in the presence of template DNA suggests strongly that this virus mutant lacks DNA polymerase. Since the cells carrying the alpha type virus are indistinguishable from other cells, it is obvious that the presence of active DNA polymerase is not essential for the maintenance of the transformed state. The observed correlation of viral DNA polymerase activity with the infectiousness of these viruses is consistent with the idea that the polymerase is essential for the initiation of host cell transformation. Since, furthermore, physically mature RSV-alpha(O) particles containing viral RNA can be formed without DNA polymerase activity, this enzyme appears to be required primarily for the integration of the viral functions into the host genome. (14 Refs)

96. ABSENCE OF POLYMERASE PROTEIN IN VIRIONS OF ALPHA-TYPE ROUS SARCOMA VIRUS.

Hanafusa H, Baltimore D, Smoler D, Watson KF, Yaniv A, Spiegelman S
Publ. Hlth. Res. Inst., New York, N.Y.
Science: 177(4055):1188-1191 1972

Noninfectious alpha-type Bryan strain Rous sarcoma virus. (RSValpha), derived from RSV-transformed chicken cells, was examined for DNA polymerase with different template-primer complexes. No DNA polymerase could be demonstrated in RSValpha in assays with poly(A).oligo(dT), poly(C).oligo(dG) or poly(A).poly(dT). In related experiments, RSValpha was tested for its ability to absorb antibody from a monospecific rat anti-avian myeloblastosis virus DNA polymerase anti-serum. RSValpha was negative in this test, indicating that the virus does not even contain DNA polymerase as a latent but immunologically active protein. Monospecific antiserum prepared against the isolate DNA polymerase of avian myeloblastosis virus (AMV) was used to study the RNA-directed DNA polymerase activity of several detergent-disrupted C-type viruses. The antiserum was able to neutralize the RNA directed DNA polymerase activity of all avian leukemia-sarcoma viruses tested irrespective of viral antigenic subtype. The DNA polymerase activity of avian reticuloendotheliosis virus and of a variety of mammalian oncornaviruses was not affected by antipolymerase antiserum. The anti-polymerase antiserum showed no activity against the seven major structural AMV proteins or normal cellular DNA polymerases. The RSValpha(O) mutant, which lacks DNA polymerase activity, showed a 60-fold decrease in the demonstrable polymerase antigen level. Indirect immunofluorescence studies showed that the viral polymerase was a cytoplasmic constituent of virus-producing chicken cells. Polymerase antigen was absent in uninfected chicken cells.

97. CHARACTERIZATION OF TWO CONDITIONAL EARLY MUTANTS OF ROUS SARCOMA VIRUS.

Linial M, Mason WS
U. Southern California Sch. Med., Los Angeles
Virology: 53(1):258-273 1973

Some of the biological and physical properties of two independently isolated mutants of Rous sarcoma virus have been investigated. Both ts 335 and ts 337 are unable either to transform or replicate at 41 C. Both viral replication and cell transformation, however, are initiated after a short time at 35 C. Once initiation has occurred, replication and transformation can be maintained after a shift to 41 C. The lesions in the mutants occur very early after infection (prior to

2 hr); however, the lesions appear to be expressed after adsorption to and penetration into the host cells. Both mutants contain temperature sensitive structural components, and ts 337 is also antigenically different from the wild type. The virions contain a polymerase activity which appears to be temperature sensitive in both the endogenous reaction and with poly rA/d(pT)10 as the exogenous template. Upon coinfection of chick fibroblasts with ts 335 or ts 337 and Rous-associated virus (RAV), genetically stable wild-type sarcoma virus is produced at a high frequency. Therefore, the information for the function(s) which is mutant in these viruses is also present in RAV. Results suggest that the lack of a DNA polymerase activity at the nonpermissive temperature prevents initiation of cell transformation and viral replication but that once initiation occurs, replication and transformation can be maintained.

98. DNA POLYMERASE ACTIVITY FROM TWO TEMPERATURE-SENSITIVE MUTANTS OF ROUS SARCOMA VIRUS IS THERMOLABILE.

Verma IM, Mason WS, Drost SD, Baltimore D
Ctr. Cancer Res., Massachusetts Inst. Tech., Cambridge
Nature (Lond): 251(5470):27-31 1974

The DNA polymerase from two temperature-sensitive mutants of Rous sarcoma virus (RSV) was purified and the activities of the purified enzymes were compared with those of the DNA polymerase of wild type RSV. The results demonstrate the covariation of thermolability of the virion DNA polymerase with temperature sensitivity of infection and transformation by the virions. The two early temperature-sensitive mutants of RSV were found to have DNA polymerase more thermolabile than that of the wild type virus, while revertants of the mutants have DNA polymerase with wild type properties. This indicates that RSV RNA encodes the information for at least a part of the DNA polymerase molecule and the enzyme must be necessary to initiate infection or transformation by RSV.

99. TEMPERATURE-SENSITIVE DNA POLYMERASE FROM ROUS SARCOMA VIRUS MUTANTS. (PP. 267-272)

Baltimore D, Verma IM, Drost S, Mason WS
Tumor Virus-Host Cell Interaction; Edited by A. Kolber.
(New York: Plenum Press) 1975

Properties of the DNA polymerase isolated from the virions of temperature-sensitive (ts) Rous sarcoma virus mutants and efforts to delineate the function of this DNA polymerase are reviewed. The enzymes from two mutants (ts335 and ts337) and from the parental wild-type Prague C Rous sarcoma virus were purified, and their heat lability was determined by incubation for various lengths of time at 44 C, followed by assay of their ability to synthesize DNA at 34 C. Both mutant enzymes were inactivated more rapidly at 44 C than the wild-type enzyme with use of an RNA template:primer combination of poly(A):oligo(dT) or a DNA template:primer, poly(dC):oligo(dG). The inactivation rate was slightly less with the DNA template. The virion DNA polymerase isolated from avian viruses is also a ribonuclease H. The activity of ribonuclease H in the ts337 enzyme was much more heat-labile than that of the wild type at 47 C; it was more stable than the DNA polymerase activity at 44 C although there was a difference between the mutant and the wild-type enzyme at that temperature. Recombinants between the mutants and wild-type virus were selected by infection of cells with the ts Rous sarcoma and a nontransforming group B leukemia virus. Two ts recombinants and two wild-type recombinants were selected and their DNA polymerase activities assayed. The ts viruses had heat-labile polymerases, while the wild-type viruses had normal polymerases. These data demonstrate that the DNA polymerase in the virions of

RNA tumor viruses is encoded by a gene of the virus. Studies on recombinants and revertants support the idea that the DNA polymerase is responsible for an early step in virus production. All these findings indicate that a virus-encoded DNA polymerase is responsible for synthesizing a DNA copy of the viral genome shortly after cell infection by RNA tumor viruses.

100. THERMOLABILE REVERSE TRANSCRIPTASE OF A MAMMALIAN LEUKEMIA VIRUS MUTANT TEMPERATURE SENSITIVE IN ITS REPLICATION AND SARCOMA VIRUS HELPER FUNCTIONS.

Tronick SR, Stephenson JR, Verma IM, Aaronson SA
Viral Carcinogenesis Branch, NCI, Bethesda, Maryland
20014

J Virol; 16(6):1476-1482 1975

Three temperature-sensitive mutants of the Rauscher strain of murine leukemia virus are defective in early post-penetration functions required both for leukemia virus infection and for initiation of transformation of cells by their pseudotypes of murine sarcoma virus. In the present study, the reverse transcriptase of one of these mutants (ts 29) is shown to be thermolabile compared with the enzymes of the wild-type virus and several other temperature-sensitive mutants. These findings provide evidence that the reverse transcriptase is required both for leukemia virus infection and for initiation of transformation by the replication-defective murine sarcoma virus genome. (Author Abstract)

101. COOPERATIVE TRANSFORMATION STUDIES WITH TEMPERATURE-SENSITIVE MUTANTS OF ROUS SARCOMA VIRUS.

Balduzzi PC

Univ. Rochester Sch. Medicine and Dentistry, Rochester, NY 14642

J Virol; 18(1):332-343 1976

Stocks of Rous sarcoma virus Bryan strain were mutagenized by a bromodeoxyuridine treatment immediately after infection. Thirty temperature-sensitive (ts) mutants defective in transformation (td) were isolated by a replica plating technique. Twenty of these mutants were preliminarily characterized and found to be defective in late functions related to transformation. These mutants were used in experiments of cooperative transformation with four Prague strain td ts mutants of different cotransformation groups. A small number of Bryan ts mutants cooperated with some of the Prague mutants in transforming chicken embryo cells at the nonpermissive temperature. However, the amount of cotransformation observed was lower than that observed with cooperating Prague ts mutants, and no clear-cut pattern of cotransformation was obtained in Prague and Bryan crosses. Indirect evidence indicates that cooperative transformation is the result of recombination events. If cooperative transformation depends on genetic homology, this type of study could supplement the results of molecular hybridization studies on the similarities in the transforming genes of different isolates of avian sarcoma viruses.

102. GROWTH STIMULATION OF CHICK EMBRYO NEURORETINAL CELLS INFECTED WITH ROUS SARCOMA VIRUS: RELATIONSHIP TO VIRAL REPLICATION AND MORPHOLOGICAL TRANSFORMATION.

Calothy G, Pessac B

Fondation Curie-Institut du Radium, Bat. 110, 91405, Orsay, France

Virology; 71(1):336-345 1976

The relationship of induction of cell multiplication to viral replication and morphological transformation was analyzed by infecting 7-day-old chick embryo neuroretinal

(NR) cells with conditional and nonconditional transformation-defective viruses. NR cells infected with a temperature-sensitive mutant of Rous sarcoma virus (RSV) defective for cell transformation, but not for virus replication, were induced to multiply at both nonpermissive and permissive temperatures, although expression of the transformed phenotype, as tested by several parameters, was suppressed in these cells at nonpermissive temperature. Nonconditional, nontransforming viruses replicated normally in NR cells, but failed to induce their multiplication. These results indicate that only transforming viruses can induce NR cell multiplication and that viral replication alone does not account for the growth changes in infected NR cells. These data also suggest that expressions of the transformed phenotype and induction of NR cell proliferation may depend on distinct viral information.

103. TRANSFORMATION-DEFECTIVE MUTANTS OF AVIAN SARCOMA VIRUSES: THE GENETIC RELATIONSHIP BETWEEN CONDITIONAL AND NONCONDITIONAL MUTANTS.

Bernstein A, MacCormick R, Martin GS

Ontario Cancer Inst., 500 Sherbourne St., Toronto, Ontario M4X 1K9, Canada

Virology; 70(1):206-209 1976

The "x segment" of the avian sarcoma viral genome is necessary for transformation of fibroblasts. Crosses between nonconditional transformation-defective mutants (lacking x segments; Rous sarcoma virus, subgroup b) and temperature-sensitive mutants (RSV, subgroup a) were made, to determine how many of the mutations resulting in temperature-sensitive transformation lie in the x segment. The results suggest that the RSV-b mutant results from a deletion close to the 3' end of the genome. Crosses between temperature-sensitive and transformation-defective mutants yielded transforming recombinants containing the entire genome. Tests of these recombinants by an infectious center technique suggest that the mutations in all of the temperature-sensitive viruses lie within the x segment.

III. VIRAL MUTATIONS SPECIFICALLY AFFECTING THE EXPRESSION OF THE TRANSFORMED PHENOTYPE

A. DNA Tumor Virus Mutations and the Expression of the Transformed Phenotype

104. TEMPERATURE-DEPENDENT PROPERTIES OF CELLS TRANSFORMED BY A THERMOSENSITIVE MUTANT OF POLYOMA VIRUS (MEETING ABSTRACT).

Dulbecco R, Eckhardt W

Salk Inst. Biol. Stud., San Diego, Calif.

Proc Natl Acad Sci USA; 67(4):1775-1781 1970

The effects of temperature-sensitive mutants of polyoma virus, ts-3 on the characteristics of BALB-3T3 cells transformed in tissue cultures were studied by the use of labeled thymidine and radioautography. There is some induction of cellular DNA synthesis in ts-3-infected cells at 39°C but the proportion of cells incorporating thymidine at 32°C is greater as is the induction of movement. Whereas topoinhibition is temperature-dependent for the ts-3-transformed cells, neither wound serum requirement nor growth in agar showed temperature-dependence. Possibly, temperature-dependent mechanisms can be controlled by the product of the ts-3 gene.

105. TEMPERATURE-DEPENDENT SURFACE CHANGES IN CELLS INFECTED OR TRANSFORMED BY A THERMOSENSITIVE MUTANT OF POLYOMA VIRUS (MEETING ABSTRACT).

Eckhart W, Dulbecco R, Burger MM
Salk Inst. Biol. Studies, San Diego, Calif.
Proc Natl Acad Sci USA; 68(2):283-286 1971

BALB/3T3 mouse cells were infected with wild type polyoma virus or with a temperature-sensitive mutant of polyoma virus (ts-3); infected cells were treated with wheat germ agglutinin in amounts of 36-180 microg/ml, and the agglutination of virus-infected cells was observed in cultures grown at 39 and 32 C. Enhanced agglutination was seen in cells infected with ts-3 and grown at 32 C but not cells infected with ts-3 and grown at 39 C. Enhanced agglutination was seen in cells infected with wild-type polyoma virus and grown at either temperature. It was found that agglutination enhancement required cellular DNA synthesis but did not require viral DNA synthesis. In a related experiment, baby hamster kidney cells transformed by ts-3 were tested for agglutination by agglutinin and concanavalin A (Con A) after being grown at 39 and 32 C. Ts-3-transformed cells showed increased agglutinability when grown at 32 C, but not when grown at 39 C. The viral gene apparently controls the surface alteration responsible for changing agglutinability in virus-transformed cells.

106. SIMIAN VIRUS 40 FUNCTIONS REQUIRED FOR THE ESTABLISHMENT AND MAINTENANCE OF MALIGNANT TRANSFORMATION.

Martin RG, Chou JY
Lab. Molecular Biology, Natl. Inst. Arthritis, Metabolism and Digestive Diseases, Bethesda, MD 20014
J Virol; 15(3):599-612 1975

Members of the five classes of temperature-sensitive simian virus 40 mutants were tested for their ability to transform Chinese hamster lung cells. Two criteria for transformation were used: the ability to form clones in medium with low serum concentrations and the ability to overgrow a monolayer. Only A mutants failed to transform at the restrictive temperature when subconfluent Chinese hamster lung monolayers were used. However, both A and D mutants failed to transform at the restrictive temperature when confluent monolayers and depleted medium were used. When transformed clones were selected, purified by recloning, and examined at both temperatures, only cell lines induced by A mutants lost the transformed phenotype at the higher temperature. Thus, A function is required for maintenance of the transformed phenotype in Chinese hamster lung cells. A function is known to be required for the initiation of viral DNA synthesis in permissive cells. Therefore, transformation may be a consequence of the introduction into a cell of the capacity for aberrant initiation of DNA replication. (Author abstract) (16 Refs)

107. SIMIAN VIRUS 40 GENE A FUNCTION AND MAINTENANCE OF TRANSFORMATION.

Osborn M, Weber K
Cold Spring Harbor Lab., N.Y.
J Virol; 15(3):636-644 1975

Temperature sensitive mutants of Simian virus 40 (SV40) were used to isolate transformants of rat embryo cells. Cells from CDF albino inbred (Charles River) rats were infected at low multiplicity (approximately 1 or less) with either wild-type SV40 or the mutant strains tsA7 or tsA28. Transformants were isolated, cloned, and examined for differences in growth properties, morphology, and expression of T-antigen at 33 and 41 C. To measure growth rate, cells were seeded in 10% fetal calf serum at 105 cells/dish and allowed to attach at 33 C.

After attachment, half of the plates were shifted to 41 C. Growth rates of both wild-type and mutant transformants were equal at 33 C, but the wild-type grew slightly faster at 41 C, while the tsA28 transformant grew slower and seemed limited to a 10- to 12-fold lower density than at 33 C. Similar results were obtained with 1% fetal calf serum. Phase microscopy showed no morphological changes in wild-type transformants with the temperature change, while the mutants became large and flat at 41 C. Size distribution also became abnormal in the mutant transformants. The size distributions of the wild-type transformants at 33 and 41 C and of the tsA transformant were similar. The distributions were normal and peaked at an arbitrary setting of 30, with only 2% of the cells being larger than 85. Four days after the shift to 41 C, however, no real peak in the tsA was seen, and 30% of the population had a size larger than 85. Staining with actin antibody showed the wild-type transformants at both temperatures, and the mutants at 33 C had few thick actin-containing fibers; the mutants at 41 C showed long, thick fibers. Of the wild-type transformants, 98% had T-antigen at both temperatures at all times tested. The mutants were also 98% positive for T-antigen at 33 C, but at 41 C the percentage decreased with time. It is concluded that wild-type and mutant transformants behave similarly at permissive temperatures, but differently at nonpermissive temperatures; this suggests that in these cells, the A function is involved in maintaining the transformed state.

108. FUNCTION OF SIMIAN VIRUS 40 GENE A IN TRANSFORMING INFECTION.

Tegtmeyer P
Dept. Pharmacol. Microbiol., Case West. Reserve Univ., Cleveland, Ohio
J Virol; 15(3):613-618 1975

The effect of Simian virus 40 (SV40) temperature-sensitive gene A on the growth characteristics of transformed cells was studied with several well-characterized A mutants in a variety of host cells. Cellular growth was quantitated by simple colony assay. When Swiss/3T3 cells were first stably transformed by A28 or A30 at 33 C and then shifted to 39 C as dilute suspensions of single cells, the cells formed colonies with a transformed phenotype as did cells transformed by a wild type virus. Rabbit kidney cells were also tested (at 33 and 41.5 C) for temperature-sensitive characteristics of transformation by wild type virus and SV40 mutants. The expression of the A gene appeared to affect the growth characteristics of rabbit cells stably transformed by A28 but not cells transformed by five other A mutants. The efficiency of colony formation by hamster embryo cells was studied after initiation of stable transformation at 33 C. Uninfected cells and cells transformed by wild type or A mutant virus were dispersed, serially diluted, and cultured at 33 or 40 C. The temperature shift decreased the capacity of the A-transformed cells to form colonies to the level of untransformed control cells. Fluorescent antibody assay for T antigen performed on each of the 3T3, rabbit, and hamster cell lines at either permissive or nonpermissive temperature showed that the antigen was present in each cell line. Since the outcome of the virus-cell interaction depended on the nature of the A mutation in a given cell species and on the species of the cell transformed by a given mutant, the transformation process may require two distinct events, each related to A gene expression.

109. ROLE OF SIMIAN VIRUS 40 GENE A FUNCTION IN MAINTENANCE OF TRANSFORMATION.

Brugge JS, Butel JS
Baylor Coll. Med., Houston, TX
J Virol; 15(3):619-635 1975

To determine whether Simian virus 40 (SV40) gene A function is involved in the maintenance of transformation,

normal cells were transformed with group A mutants and were examined after growth at the permissive and nonpermissive temperatures. Morphology, saturation density, colony formation (on plastic, monolayers of normal cells, and in soft agar), 2-deoxy-D-glucose uptake, and expression of SV40 tumor and surface antigens were all studied. Multiple parameters were monitored to determine whether loss of the SV40 gene A function affected all, several or none of the characteristics of the transformed state. SV40 group A mutants A7, A28, A30 and A58, the wild type 2 and the wild type Baylor reference strains were used. Normal cells consisted of human skin cells derived from a patient with Fanconi anemia, hamster embryo fibroblast cells, and BALB/3T3 mouse cells. The group A mutant-transformed cells grown under permissive temperature formed multiple cell layers and cells with a rounded morphology; under nonpermissive conditions, the cells were fibroblastic and highly contact-inhibited, resembling the morphology of normal cells. When cells were shifted back to the permissive temperature, they returned to the original transformed morphology within two days. Wild type-transformed cells of hamster, human, and mouse showed the same morphology at both temperatures. Group A mutant-transformed cells paralleled the rapid growth and high saturation densities of wild type-transformed cells under permissive conditions. At the nonpermissive temperature, these cells showed the slower growth rates and lower final saturation densities of normal cells. A temperature-dependent variation in colony formation was observed with mutant-transformed cells. Cells from all three species formed 4- to 51-fold fewer colonies at the nonpermissive temperature than at the permissive temperature on plastic, and 4- to 18-fold less on normal cell monolayers and on soft agar. Temperature-shift studies with normal and transformed hamster cells seeded into plastic Petri dishes indicated that the apparent reversion of A-mutant-transformed cells to a normal phenotype is a reversible event. Both hexose uptake and expression of SV40-induced antigens were temperature-dependent. It concluded that the continual expression of the gene A function is required for maintenance of transformation.

110. INITIATION AND MAINTENANCE OF CELL TRANSFORMATION BY SIMIAN VIRUS 40: A VIRAL GENETIC PROPERTY (MEETING ABSTRACT).

Kimura G, Itagaki A

Tottori Univ. Sch. Med., Yenago 683, Japan

Proc Natl Acad Sci USA; 72(2):673-677 1975

The role of Simian virus 40 (SV40) genes in biological phenomena in cells after infection and transformation by this virus was studied. The transforming ability in 10% serum medium of the temperature-sensitive mutants of SV40 in the complementation group III (ts610 type mutants) was greatly reduced when infected rat 3Y1 cells were incubated at the restrictive temperature of 40 C or incubated first at 40 C for three days and then shifted to the permissive temperature of 33 C. Transformation did occur efficiently after incubation at 33 C, or after an initial incubation at 33 C for five days followed by a shift to 40 C. When growth properties of 3Y1 cells transformed at 33 C by the group III mutants were examined at 40 C, several aspects of the transformed state were rendered temperature-sensitive. These aspects were the ability of cells to grow in low serum (1.5%) medium and to make colonies, in 10% serum medium, on monolayers of untransformed 3Y1 cells and in soft agar. It is concluded that a Simian virus 40 gene (cistron III) controls the initiation, as well as at least some aspects of the maintenance, of transformation and that the initiation reaction is a more heat-labile event than the maintenance reaction(s) under the experimental conditions.

111. SV40 TRANSFORMATION OF MOUSE BRAIN CELLS: CRITICAL ROLE OF GENE A IN MAINTENANCE OF THE TRANSFORMED PHENOTYPE.

Anderson JL, Martin RG

Natl. Inst. Arthritis, Metabolism, Digestive Diseases, Natl.

Insts. Health, Bethesda, MD 20014

J Cell Physiol; 88(1):65-76 1976

Brain cells derived from the NIH Swiss mouse strain were established in tissue culture. This strain was chosen because it was shown to be free of mouse-tropic AKR-type murine leukemia viruses. Astrocytic neuroglial cells, identified by morphology and staining properties, predominated. The brain cell culture was successfully transformed with SV40 wild-type virus and with a representative early (A239) and late (C219) mutant. When subjected to growth analysis, the A239 transformant displayed selective loss of six characteristics of the transformed phenotype at the restrictive temperature (40.5 C): doubling time, saturation density, ability to grow in low serum, efficiency of growth on plastic and on normal brain cell layers, and cloning in soft agar. Temperature shift experiments demonstrated the reversibility of the differences in saturation density. T-antigen was expressed at 33 C and 40.5 C. Alteration in uptake of 2-deoxyglucose was not a characteristic of the transformed phenotype. The cell lines may be of use in brain culture work and in studies on the mechanism of SV40 transformation.

112. TEMPERATURE-DEPENDENT PROPERTIES OF CELLS TRANSFORMED BY A THERMOSENSITIVE MUTANT (TS121) OF POLYOMA VIRUS.

Hakura A

Res. Inst. Microb. Dis., Osaka Univ., Japan

Biken J; 17(3):117-119 1974

The properties of cells transformed by the temperature sensitive mutant of polyoma virus, ts-121, that seem to control replicating ability in soft agar, are described. Ts-121 was isolated by hydroxylamine treatment from plaque purified SE-polyoma virus strain 105. The plaque forming ability of ts-121 was 10-4 or less at 39 C than at 35 C. A31-714 cells (recloned cell line from BALB/3T3 mouse) were transformed with UV-irradiated ts-121, and several independent cell lines were isolated. Each of the transformed cell lines was seeded onto six plates in 0.33% agar. Three replicate plates were incubated at 39 C for 30 days and the other three were incubated at 35 C for 40 days. All the transformed cell lines induced by UV-ts-121, except one, showed 12-20 times higher efficiency of colony formation at 35 C than at 39 C. Three cell lines transformed by the wild type virus showed similar efficiencies of colony formation at 35 C and at 39 C under the same conditions. These results indicate that the colony forming ability of the ts-121-transformed lines in soft agar was affected by temperature. Two possible explanations are offered for this low efficiency of colony formation at high temperatures: 1) the growth of these cells in soft agar may be temperature dependent; or 2) the low efficiency of colony formation at 39 C may be due to death of transformed cells at high temperature. The first explanation is taken as the more likely one because the ability of ts-121-transformed cells to grow to the contact-inhibited state in liquid culture is completely temperature dependent and cells cultivated in the contact-inhibited state still retain their replicating ability at 35 C after incubation for 25 days at 39 C. These observations suggest that the ability of transformed cells to grow in soft agar is closely related to the function of the ts-121 gene. The role of ts-121 gene in the regulation of growth of transformed cells in soft agar, however, is unknown.

113. TEMPERATURE-DEPENDENT PROPERTIES OF CELLS TRANSFORMED BY A THERMOSENSITIVE MUTANT (TS-121) OF POLYOMA VIRUS. II. CHARACTERIZATION OF 121-6 CELLS.

Okada YS, Hakura A

Res. Inst. for Microbial Diseases, Osaka Univ., Suita,
Osaka, Japan

Int J Cancer; 16(3):394-403 1975

The growth and agglutinability properties of a cell line transformed by a temperature-sensitive mutant of polyoma virus (ts-121) in liquid media were studied. The cell lines used were: A31-714, a recloned cell line from BALB/3T3 cells; PV-4, a line of A31-714 cells transformed by wild-type polyoma virus; and 121-6-5, a subclone isolated from 121-6 (a transformed line induced by ts-121). Determinations were made of cell density and agglutinability by concanavalin A (Con A); autoradiography was performed after treatment with 5 microCi/ml of 3H-thymidine. The cells were grown at 35 or 39 C. Both the morphology and saturation density of the 121-6 cells were affected by temperature; at 39 C, they grew to monolayer sheets and remained contact inhibited for at least 25 days, while at 35 C, they grew beyond the monolayer density, thus resembling the PV-4 cells. When the cells were incubated at 39 C and the temperature was shifted to 35 C before the cells became confluent, they continued to grow beyond the saturation density normally reached at 39 C. However, when they were downshifted after reaching confluence, they began to grow only after a 3-4 day latent period. When sparse cultures were moved from 35 to 39 C, they continued to grow to form a monolayer; when cultures which had grown beyond confluence were similarly upshifted, growth stopped and a gradual decrease in cell density was observed. The agglutinability of the 121-6-5 cells was not temperature-dependent; even at high temperatures, these cells showed a high agglutinability. The data suggest that the agglutinability by plant lectin may not be directly correlated with other phenotypic markers of transformants, such as the changes in cell morphology and saturation density.

114. VIRAL CARCINOGENESIS.

Rosen P

Dept. Physics and Astronomy, Univ. Massachusetts,
Amherst, MA 01002

J Theor Biol; 64(2):215-220 1977

A mathematical expression for transformation probability is derived using the assumption that the A gene of an oncogenic, temperature-sensitive mutant polyoma virus makes an A protein that binds to a regulator gene to cause transformation. If the regulator gene is the operon controlling divisional proteins, its binding to A protein will prevent repressor protein from being synthesized, resulting in uncontrolled mitosis. The probability after long times is the concentration of the A protein (CA) divided by the dissociation constant (KD). Calculations were compared to experimental data obtained by measuring transformation frequencies at 31.5 C as a function of virus concentration. Under proper temperature conditions, dissociation can occur. At 31.5 C, a reasonable estimate for KD is 10^{11} -7; at 38.5 C, transformation is very improbable. (4 Refs)

B. RNA Tumor Virus Mutations and the Expression of the Transformed Phenotype

115. ROUS SARCOMA VIRUS: A FUNCTION REQUIRED FOR THE MAINTENANCE OF THE TRANSFORMED STATE.

Martin GS

Virus Lab., U. California, Berkeley

Nature (Lond); 227(5262):1021-1023 1970

The isolation of a temperature-sensitive mutant of the Schmidt-Ruppin strain of Rous sarcoma virus, the properties of which indicate that mutation affects a function required for the maintenance of the transformed state but not for growth of the virus, is described. Survivors of a crude stock of SR-RSV-A virus which had undergone mutagenic treatment with N-methyl-N'-nitro-N-nitrosoguanidine (2 mg/ml) were cloned at 35-36C using an agar suspension of chick fibroblasts. One of the 6 mutants isolated which were able to form foci at 36C but not at 41C was selected for study. The rate of virus accumulation and the rate of inactivation was the same for both the mutant and the wild type at 41C, indicating that mutation did not affect the growth of the virus. When T1-transformed cells (at 36C) were shifted to 41C, the cells rapidly lost their rounded refractile appearance and assumed a near-normal appearance within 4 hr; cells infected by the wild type virus were unaffected by the temperature shift. The reverse change, when T1-infected cells grown at 41C were shifted to 36C and became completely transformed, was slower, taking about 2 days. Part of the viral genome required for maintenance of the transformed state is apparently lost or inactivated at the nonpermissive temperature in this mutant, although its growth is not affected.

116. A MUTANT OF ROUS SARCOMA VIRUS (TYPE O) CAUSING FUSIFORM CELL TRANSFORMATION (MEETING ABSTRACT).

Yoshii S, Vogt PK

Nagoya U. Sch. Med., Japan

Proc Soc Exp Biol Med; 135(2):297-301 1970

Chick embryo fibroblasts were infected with a virus derived from Rous associated virus which produced some foci with fusiform cell morphology, and this fusiform-transforming virus was subsequently isolated. The fusiform-transforming virus had the envelope properties of a strain of Rous sarcoma virus which causes round cell transformation and had an identical host range producing infections in chicken cells, in Japanese quail cells, and in pheasant cells. Three serum antigens which neutralize the infectivity of the round cell-transforming Rous sarcoma virus also neutralized the infectivity of the fusiform-transforming virus strain at the same titers required for round cell-transforming virus neutralization. Apparently, the fusiform-transforming and round-cell transforming viruses are mutants of the same virus.

117. THE EFFECTS OF RECIPROCAL CHANGES IN TEMPERATURE ON THE TRANSFORMED STATE OF CELLS INFECTED WITH A ROUS SARCOMA VIRUS MUTANT.

Kawai S, Hanafusa H

Public Hlth. Res. Inst., City New York, N.Y.

Virology; 46(2):470-479 1971

This paper describes the basic characteristics of a temperature-sensitive mutant virus and the behavior of cells infected with the virus under various physiologic conditions. Mutagenized viruses were obtained from stock Schmidt-Ruppin strain Rous sarcoma virus by treatment with 5-fluorouracil and then inoculated into C/O or C/O' type chick embryo cell cultures. Cloning studies on the infected embryo cells indicated that five separate clones could produce

temperature-sensitive transformed cells. One such virus, Ts-68, was isolated and used for specific experimental studies, along with the wild type virus; tests were conducted at 37 and 41 C. Wild-type virus could induce focus transformation and colony formation at both temperatures, and could readily induce tumors when it was given to chickens (body temperature approximately 41 C) *in vivo*. On the other hand, Ts-68 was highly temperature-dependent. The cells maintained at 37 C behaved like the wild-type, but at 41 C focus formation was inhibited, cell growth was greatly reduced, and tumor production *in vivo* was both suppressed and delayed; in addition, sugar consumption from the media by infected cells was reduced and the rate of virus production was initially slowed. These aberrant conditions, however, were all found to be reversible when the cells were incubated at 37 C; the cells took on the transformed appearance and behaved, for all practical purposes, like the wild-type cells and like the cells continuously incubated at 37 C. It was also discovered that biochemical inhibitors, particularly puromycin and cycloheximide, could inhibit transformation shift upon temperature change and that 37 C-maintained cells could revert to the 41 C type if the media was unchanged for a long period. It is therefore postulated that a virus-coded, nonstructural protein which is temperature sensitive plays an essential role in cell transformation. This heat-sensitive protein formed in the mutant-infected cells may be somewhat unstable and, consequently, continuous synthesis of this molecule would be required to maintain the transformed state.

118. INDUCTION OF MUTATIONS IN AN RNA TUMOR VIRUS BY AN ANALOGUE OF A DNA PRECURSOR.

Bader JP, Brown NR
Natl. Cancer Inst., Bethesda, Md.
Nature New Biol; 234(44):11-12 1971

The effect of 5-bromodeoxyuridine (BrdU) on the infectivity and transforming ability of the transforming Bryan strain of Rous sarcoma virus (RSV), the nontransforming Rous-associated virus (RAV) and phenotypic mixtures, was studied in chick embryo cells. Infectivity, determined by the number of focus-forming units, was decreased only if BrdU (25-100 microg/ml) was present during the first 12 hr immediately after exposure of the cells to virus. RSV yields from BrdU-treated cultures showed a 1.3- to 1.9-fold increase in infectivity at low temperatures (36 C) and a slight decrease at high temperatures (40.5 C), as compared to controls, indicating that a significant proportion of the virions produced by BrdU-treated cells containing a genetic defect(s) which caused a temperature-sensitivity for the transformation process. Established virus-producing cells treated with BrdU did not produce such temperature-sensitive populations, nor did cells pretreated with BrdU before infection. With increasing time after infection of cells and exposure to BrdU, the proportion of the general virion population consisting of temperature-sensitive transforming virions decreased. Attempts to isolate RSV defective in the genomic regions responsible for transformation resulted in cells which produced both RAV and temperature-sensitive RSV. Of 119 transformed clones isolated from foci induced by BrdU-RSV, 20 yielded RSV which failed to induce transformation at 40.5 C. Seven of these latter clones produced RSV which transformed cells only at 36 C; the other 13 clones produced virus which did alter cell morphology at 40.5, but only when infection was made using high multiplicities per cell. These morphological changes were atypical of the transformation characteristic of the Bryan 'high titer' strain of RSV. No temperature-sensitive RSV was found in clonal isolates of control RSV-induced foci. The production of mutations in RSV, an RNA tumor virus, by BrdU demonstrated that the

replicative form of the virus, at least the region determining transformation, was DNA.

119. TEMPERATURE-DEPENDENT TRANSFORMATION OF CELLS INFECTED WITH A MUTANT OF BRYAN ROUS SARCOMA VIRUS.

Bader JP
Natl. Cancer Inst., Bethesda, Md.
J Virol; 10(2):267-276 1972

Chick embryo cells infected with a mutant (Ta) of the Bryan high-titer strain of Rous sarcoma virus (RSV-BH) are morphologically transformed at 36 C but appear similar to uninfected cells at 41 C. When cells infected with RSV-BH-Ta are switched from 41 to 36 C, morphological changes characteristic of transformation are observable within 10 min. The transformation is reversible; cells shifted from 36 to 41 C have been observed to lose their transformed morphology within 1 hr. The transformation after a shift in temperature is unaffected by inhibition of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or protein synthesis, demonstrating that the proteins involved in the morphological change are already present. Transformed cells infected with RSV-BH or RSV-BH-Ta take up hexose and synthesize hyaluronic acid at higher rates than uninfected cells or RSV-BH-Ta-infected cells grown at 41 C. However, inhibition of either protein or RNA synthesis, but not DNA synthesis, prevented the induction of increased hexose uptake and hyaluronic acid synthesis after a shift of RSV-BH-Ta-infected cells from 41 to 36 C. Therefore, these biochemical changes are secondary to a more basic change responsible for morphological transformation.

120. CHARACTERISTICS OF A CONDITIONAL MUTANT OF ROUS SARCOMA VIRUS DEFECTIVE IN ABILITY TO TRANSFORM CELLS AT HIGH TEMPERATURE.

Biquard JM, Vigier P
Institut du Radium, Faculte des Sciences, 91-Orsay,
France
Virology; 47(2):444-455 1972

Mutagenesis with 5-fluorouracil was used to obtain a conditional mutant (FU-19) of Rous sarcoma virus (RSV) with a highly reduced ability to transform chicken cells at 41 C; the parent RSV strain was SR4. FU-19 and SR4 were compared with respect to growth characteristics in chick embryo cell monolayers at 37 C and 41 C. FU-19 retained its capacity to replicate at 41 C, and virus production per infected cell was comparable for both strains; but the number of infective centers increased more slowly after low multiplicity infection than after high multiplicity infection by FU-19. When monolayer cultures of chick embryo cells were infected with FU-19 at 37 C for 1 hr and then incubated at 41 C, the number of foci of Rous cells (transformed cells) was reduced 30- to 50-fold compared to controls infected and incubated at 37 C. Additional evidence that the residual transforming ability of FU-19 may be dependent on multiplicity of infection. Cells infected and grown at 41 C show a rapid and nearly synchronous transformation to Rous cell foci within a few hr after shift-down to 37 C; cells infected and grown at 37 C 'detransformed' (showed disappearance of most Rous cell foci) within a few hr of a shift-up to 41 C. Hence, the maintenance of the transformed state requires the continuous expression of some viral gene(s). The addition of high mol wt dextran sulfate (DS) to the medium considerably increased the number of foci produced by FU-19 either residually at 41 C or by shift to the restrictive temperature; presumably FU-19 is a 'leaky' mutant, expressing transforming capacity weakly at 41 C, and this capacity is assisted by DS just as it is able to enhance transformation by normal RSV. By the use of various inhibitors of macromolecular synthesis, it was demonstrated

that protein synthesis (but not DNA or RNA synthesis) are required for transformation, whereas the macromolecular synthesis is required for detransformation. Finally, FU-19 shows the same temperature sensitivity for transformation *in vivo* as *in vitro*: it fails to produce tumors on chick chorioallantoic membrane (CAM) at 41 C, and sarcoma cells produced in CAM at 37 C disappear upon shift to 41 C. (25 Refs)

121. ISOLATION OF TEMPERATURE-SENSITIVE MUTANTS OF MURINE SARCOMA VIRUS.

Scolnick EM, Stephenson JR, Aaronson S
NCI, Bethesda, MD
J Virol; 10(4):653-657 1972

Kirsten murine sarcoma viruses mutagenized by bromodeoxyuridine or azacytidine were used to infect mouse cells. Three temperature-sensitive (ts) mutant virus cell lines were recovered from the infected cells. The ts mutants did not spontaneously release virus and did not show murine or rat viral group-specific antigens when grown at 39-40 C. The temperature sensitivity of the transformed morphology of the three ts mutant lines was due to temperature sensitivity of a viral rather than a cellular gene product. The ts mutant virus-transformed cells formed colonies on cell monolayers at 32 C but not at 39-40 C. Superinfection with Kirsten virus restored colony-forming ability at 39-40 C in one of the three ts mutant lines.

122. CONTROL OF GENE EXPRESSION IN RAT CELLS TRANSFORMED BY A COLD-SENSITIVE MURINE SARCOMA VIRUS (MSV) MUTANT.

Somers KD, May JT, Kit S
Baylor Coll. Med., Houston, Texas
Intervirology; 1(3):176-184 1973

The control of murine sarcoma virus (MSV) genetic information in rat cells transformed by a cold-sensitive MSV mutant was examined. Expression of the transformed phenotype by cells after a shift from the non-permissive (33 C) to the permissive temperature (39 C) was not prevented by inhibitors of protein and glycoprotein synthesis. Similar amounts of viral-specific RNA, measured by RNA-DNA hybridization, were found in cells grown at the permissive or nonpermissive temperature. The results suggest that the expression of transformation involves translational and possibly post-translational, but not transcriptional control.

123. TRANSFORMATION-DEFECTIVE VIRUS MUTANTS IN A CLASS OF MORPHOLOGIC REVERTANTS OF SARCOMA VIRUS TRANSFORMED NONPRODUCER CELLS.

Greenberger JS, Anderson GR, Aaronson SA
Natl. Cancer Inst., Bethesda, Md.
Cell (Camb); 2(4):279-286 1975

Following mitomycin C mutagenization of Murine Sarcoma Virus (MSV)-nonproductively transformed cells, revertants can be isolated which exhibit normal growth and yet contain sarcoma virus. Since the revertants are as susceptible as normal cells to exogenous MSV infection, it was hypothesized that they arose from mutation of the integrated viral genome rather than from a cellular alteration. Morphologic revertant clones were isolated by methylcellulose selection technique. Transformation is blocked in the revertants, and hybridization assay revealed neither a quantitative nor a qualitative defect in viral RNA transcription that accounted for this block. Following rescue with Type C helper virus, the revertants released low levels of wild-type transforming virus and excessive levels of transformation-defective sarcoma virus. Focus formation assay indicated that the defective viruses can be transmitted to new cells in the absence of morphologic alteration. These results all indicate that the revertants contain mutant viruses

defective in transforming functions. Incubation of revertant clones at 31 C, 37 C, and 39 C for up to seven days indicated that the defect(s) in the mutants were not temperature dependent. When each revertant line was infected with virus rescued from the other lines, no complementation was demonstrated. The spontaneous reappearance of transformed cells in a revertant clone is apparently concomitant with the spontaneous appearance of wild-type virus. Thus, the mutation is not a deletion. The isolated viral mutants are apparently the first obtained by mutagenization of a stably integrated viral genome in eucaryotic cells. This experimental system could be useful in identifying agents that affect the frequency of mammalian cell mutation and possibly influence the occurrence of tumors.

124. DISTINGUISHABLE TRANSFORMATION-DEFECTIVE PHENOTYPES AMONG TEMPERATURE-SENSITIVE MUTANTS OF ROUS SARCOMA VIRUS.

Becker D, Kurth R, Critchley D, Friis R, Bauer H
Institut für Virologie, Fachbereich Humanmedizin, 6300
Giessen, Federal Republic of Germany
J Virol; 21(3):1042-1055 1977

Eight transformation-defective, temperature-sensitive (ts) mutants of the Prague strain of Rous sarcoma virus, subgroup A, have been isolated after mutagenesis with 5-bromodeoxyuridine followed by selection on the basis of focus tests. Five of these mutants, ts GI201, GI202, GI203, GI204, and GI205, exhibit properties like most previously reported isolates in that they show a temperature-sensitive response to each of a variety of transformation-specific parameters tested. Interestingly, GI201, in addition to the temperature-sensitive defect, carries a lesion that was observed as a nonconditional loss of expression of plasminogen activator protease. Three mutants, ts GI251, GI252, and GI253, have been designated partial transformation-defective (PTD) mutants since they behave as ts mutants according to some tests for transformation and as wild type according to others. These three mutants fail to form foci at the nonpermissive temperature (41 degrees C) and are nontumorigenic in 3-week-old chickens (body temperature, 42 degrees C). The agglutinability by concanavalin A of cells infected with these mutants shows a definite temperature sensitivity, as do the rate of 2-deoxyglucose uptake and the disappearance of the 250,000-dalton normal cell glycoprotein (large, external, transformation sensitive (LETS)). Although the PTD mutant-infected cells, unlike cells infected with other transformation mutants, exhibit a cell-bound plasminogen activator protease at the nonpermissive temperature, this activator is not detectable as a free protease in the medium, as it is with wild-type, virus-infected cells. The PTD mutants behave like the wild-type parent in their ability to induce transformed growth properties in the infected cells, ie, growth beyond normal cell saturation density with or without serum-supplemented medium and growth leading to colony formation in soft-agar or methyl cellulose-containing suspension media. (48 Refs). (Author Abstract)

125. DIFFERENTIAL INDUCTION OF TUMOUR ANTIGENS BY TRANSFORMATION-DEFECTIVE VIRUS MUTANTS

Kurth R
Imperial Cancer Res. Fund Lab., P.O. Box 123, Lincoln's
Inn Fields, London, WC2A3PX, England
J Gen Virol; 28(2):167-177 1975

Normal rat (outbred Osborn-Mendel strain) kidney cells infected by a variety of transformation-defective temperature-sensitive avian leukosis sarcoma virus mutants were tested for the expression of transformation characteristics at permissive and restrictive temperatures. Morphology, growth behavior and agglutinability by concanavalin A corresponded fully to

the phenotype of the infected cells, at permissive temperature the cells resembled wild type virus transformed cells, whereas when grown under restrictive conditions they became virtually indistinguishable from normal cells. The quantitative expression of allo- or xenogeneic cell surface antigens was not significantly affected by the phenotype of the cells. Two out of the five tested mutants induced tumor antigens in the expected temperature-dependent manner, whereas the other three mutants were able to induce tumor-specific cell surface antigens even in the revertant cells cultured at the restrictive temperature. These findings extend previous results about tumor antigen induction in mutant-infected cells of the natural host, the chicken embryo fibroblasts. The value of transformation-defective tumor antigen-positive mutants for vaccination purposes is discussed.

126. TUMOR PRODUCING CAPACITY OF TEMPERATURE SENSITIVE MUTANTS OF AVIAN SARCOMA VIRUSES IN CHICKS.

Toyoshima K, Owada M, Kozai Y
Res. Inst. Microbial Dis., Osaka U., Japan
Biken J; 16(3):103-110 1973

Tumor production by five temperature sensitive (ts) mutants of avian sarcoma virus was tested to examine the relation between temperature sensitivity of cell transformation in vitro and the tumor-producing capacity in vivo. When ts mutants were inoculated into the wing webs of chicks, the incidence of tumors was low. However, when the inoculated chicks were exposed to unfavorable conditions during the first wk of infection, which lowered the temperature of the inoculated site, tumors appeared at high frequencies and grew rapidly. These results suggest that mutants which possess ts characters of cell transformation in vitro also have a tumor-producing capacity in vivo which is affected by temperature. Some, though not all, of the tumors produced in vivo by ts mutants contained back mutants.

127. ONCOGENICITY OF NON-TRANSFORMING MUTANTS OF AVIAN SARCOMA VIRUSES.

Biggs PM, Milne BS, Graf T, Bauer H
Houghton Poultry Res. Station, Huntingdon, England
J Gen Virol; 18:399-403 1973

Two non-transforming mutants of Rous sarcoma virus, one (NC-SRV-H) originated by point mutation and the other (NC-SRV-I) by deletion, were studied for their oncogenicity in leukosis-sarcoma virus-free Brown and White Leghorn chicks. One-day-old chicks were inoculated into the wing web ip with one of the two virus preparations and were thereafter examined daily for wing-web tumors. No sarcomas were observed in the wing webs of either strain of chick, indicating that both non-transforming mutants had lost their ability to produce tumors characteristic of their parent viruses. Although no wing-web tumors arose in treated Brown Leghorn chicks, the incidence of tumors in White Leghorn chicks was 36.6% following inoculation with NC-SRV-I and 53.5% with NC-SRV-H. The tumors consisted primarily of erythroid leukosis and lymphoid leukosis. Virus isolated from one NC-SRV-I and two NC-SRV-H-induced lymphoid tumors were non-transforming in cell culture, and, based on interference tests, each belonged to the same subgroup as the mutant which produced the respective tumor. The ability of these non-transforming mutants of Rous sarcoma virus to produce tumors characteristic of leukosis viruses suggests there is more than one gene in the genome of leukosis/sarcoma viruses responsible for neoplasia and also indicates that leukosis viruses can originate from sarcoma viruses through loss of genetic material.

128. ONCOGENICITY OF AVIAN LEUKOSIS VIRUSES OF DIFFERENT SUBGROUPS AND OF MUTANTS OF SARCOMA VIRUSES.

Purchase HG, Okazaki W, Vogt PK, Hanafusa H,
Burmester BR, Crittenden LB
Regional Poultry Research Laboratory, Agricultural
Research Service, East Lansing, Michigan 48823.
Infect Immun; 15(2):423-428 1977

Leukosis viruses of seven subgroups were tested for oncogenicity in chickens susceptible to virus infection and to development of lymphoid leukosis (LL) tumors. All subgroup A viruses and the subgroup B virus tested produced a high incidence of LL and other related neoplasms. Viruses of subgroup C and RAV-61 of subgroup F produced a low level of LL. The RAV-50 of subgroup D produced osteopetrosis. In these tests, the viruses of subgroup E and G and one virus of subgroup F were not pathogenic, possibly because infection was not established in the chickens, the chickens were not susceptible to tumor development by these viruses, or the viruses lacked oncogenicity. All temperature-sensitive mutants of Rous sarcoma virus produced sarcomas, but the level varied. One nontransforming mutant produced sarcomas, and the other three tested produced LL. All three mutants that cause cells to grow as colonies in agar produced a high incidence of sarcomas. Thus, sarcoma viruses, by back-mutation, may lose the ability to transform cells in vitro, to make cells grow in agar colonies, or to induce sarcomas in vivo, yet they retain the ability to produce LL. Conversely, it was previously shown that leukosis viruses may be changed into viruses that transform cells in vitro and produce sarcomas in vivo by suitable passage in chicks. (Author Abstract)

IV. HOST CELL MUTATIONS OR VARIATIONS AFFECTING THE INTERACTION WITH TUMOR VIRUSES

A. Reversion of Transformed Cell Properties

129. REVERSION OF PROPERTIES IN CELLS TRANSFORMED BY POLYOMA VIRUS.

Rabinowitz Z, Sachs L
Weizmann Inst. Sci., Rehovot, Israel
Nature (Lond); 220(5173):1203-1206 1968

After cultivation on irradiated feeder cultures (rat embryo cells) or glutaraldehyde-fixed normal or polyoma virus (PV)-transformed hamster embryo cells, several clones of PV-transformed hamster cells, in which the transformed state had become hereditary, showed a high frequency of variants with a reversion of the characteristic properties of transformation. This reversion was not accompanied by a loss of the PV genome.

130. THE FORMATION OF VARIANTS WITH A REVERSION OF PROPERTIES OF TRANSFORMED CELLS. 1. VARIANTS FROM POLYOMA-TRANSFORMED CELLS GROWN IN VIVO.

Rabinowitz Z, Sachs L
Weizmann Inst. Sci., Rehovot, Israel
Virology; 38(2):336-342 1969

Hamster embryo cells transformed by polyoma virus (PV), inoc sc into 6-week-old hamsters, produced tumors histologically similar to variants formed in vitro, which differed from the original transformed cells in morphology, and in that they could form colonies on glutaraldehyde fixed normal cells, had a decreased cloning efficiency in fluid medium or soft agar, failed to multiply at 41 C, and retained contact inhibition between both themselves and their parent cells. Both in vivo and in vitro variants had PV-induced

nuclear tumor (T) antigen, and the in vivo variant had a higher degree of tumorigenicity than the parent cells.

131. THE FORMATION OF VARIANTS WITH A REVERSION OF PROPERTIES OF TRANSFORMED CELLS. II. IN VITRO FORMATION OF VARIANTS FROM POLYOMA-TRANSFORMED CELLS.

Rabinowitz Z, Sachs L
Weizmann Inst. Sci., Rehovot, Israel
Virology; 38(2):343-346 1969

Polyoma virus (PV)-transformed hamster embryo cells were subcultured every 2-3 days in Eagle's medium by seeding 5×10^5 cells per 50 mm dish. After an initial decrease at 14 days, a continued increase in the percentage of variant colonies was seen, identified by their ability to form colonies on glutaraldehyde-fixed normal hamster cells. Subculture at different cell densities (2×10^4 , 2×10^5 or 2×10^6) indicated that subculture at the lowest density gave the highest frequency of variants.

132. THE FORMATION OF VARIANTS WITH A REVERSION OF PROPERTIES OF TRANSFORMED CELLS. III. REVERSION OF THE STRUCTURE OF THE CELL SURFACE MEMBRANE.

Inbar M, Rabinowitz Z, Sachs L
Weizmann Inst. Sci., Rehovot, Israel
Int J Cancer; 4(5):690-696 1969

The formation of variants from polyoma virus (PV)-transformed hamster embryo cells with a reversion of in vitro properties of transformation is associated with a partial or complete loss of agglutinability by the carbohydrate-binding protein concanavalin A (Con A). Treatment of variants with trypsin resulted in restoration of previously-lost agglutinability. Since the formation of variants was not associated with a loss of ability to synthesize the virus-specific nuclear tumor (T) antigen, it is suggested that the synthesis of T antigen is insufficient to prevent reversion of cell surface architecture. Compared with parental transformed cells, variants with a partial or complete loss of agglutinability showed the same decrease in cloning efficiency in fluid medium and soft agar and in saturation density. Apparently both complete and partial reversion of Con A sites to the cryptic form can account for the same degree of reversion of in vitro properties. It is concluded that there is some minimal number of exposed sites for Con A which must be exceeded for the cells to express the properties of transformation in vitro.

133. THE FORMATION OF VARIANTS WITH A REVERSION OF PROPERTIES OF TRANSFORMED CELLS. IV. LOSS OF DETECTABLE POLYOMA TRANSPLANTATION ANTIGEN.

Rabinowitz Z, Sachs L
Weizmann Inst. Sci., Rehovot, Israel
Virology; 40(2):193-198 1970

Variant cells, produced in polyoma virus (PV)-transformed hamster embryo cell cultures, and which were less tumorigenic than the parental cells, demonstrated a complete loss of PV transplantation antigen when no immunity against the variants was induced after immunization of hamsters with $1-2 \times 10^6$ PFU ip. Some transplantation resistance was induced by variant cells with a high tumorigenicity that were produced in vivo. The variants contact inhibited each other and inhibited the parental cells.

134. CONTROL OF THE REVERSION OF PROPERTIES IN TRANSFORMED CELLS.

Rabinowitz Z, Sachs L
Weizmann Inst. Sci., Rehovot, Israel
Nature (Lond); 225(5228):136-139 1970

When hamster embryo cells were transformed by polyoma virus and seeded for cloning at various densities, the frequency of variant cells possessing certain reverted characteristics was highest at low density (3×10^2 cells/5-cm Petri dish). The variants re-reverted to transformed cells during subsequent cell crowding; the re-reversion could be prevented by inhibiting cell multiplication. The variant cells contained a higher number of chromosomes than did the transformed cells.

135. THE FORMATION OF VARIANTS WITH A REVERSION OF PROPERTIES OF TRANSFORMED CELLS. VI. STABILITY OF THE REVERTED STATE.

Rabinowitz Z, Sachs L
Weizmann Inst. Sci., Rehovot, Israel
Int J Cancer; 9(2):334-343 1972

The stability of the reverted state was analyzed in revertants from polyoma-virus-transformed hamster cells in which reversion was not associated with a loss of the viral genome. The frequency of reversion was studied in 12 cloned revertants after different periods of in vitro cultivation. The regaining of two transformed properties, the ability to form colonies at 41°C and in soft agar, were used as markers for reversions. No temperature re-revertants and 2 to 184 agar re-revertants per 5×10^4 cells were detected 45 days after isolation of the clones. At 90 days after isolation, there were 14 to 784 temperature re-revertants and 242 to 1160 agar re-revertants per 5×10^4 cells. Thus, the stability of the reverted state was decreased by in vitro cultivation. A study of the isolated revertants indicated that once a transformed property has been regained during re-reversion, the immediate progeny of the re-revertants may contain a high frequency of segregants which had lost this regained property. The re-reverted state was more stable in re-revertants isolated from revertants 100 days after cultivation than in re-revertants from revertants cultured for 55 days. Reversion for one transformed property was not necessarily associated with re-reversion for the other transformed property. The different degrees of stability of the reverted state could possibly be explained by differences in the presence of chromosomes responsible for either expression or suppression of the transformed properties.

136. THE FORMATION OF VARIANTS WITH A REVERSION OF PROPERTIES OF TRANSFORMED CELLS. VII. IN VITRO LIMITED LIFE SPAN OF VARIANTS ISOLATED FROM TUMORS.

Rabinowitz Z, Sachs L
Weizmann Inst. Sci., Rehovot, Israel
Int J Cancer; 10(3):607-612 1972

Tumors were induced in hamsters following the sc inoculation of hamster embryo cells transformed in vitro by dimethylnitrosamine (DMNA). Cultures which were subsequently established from these tumor cells showed morphological and growth properties which differed from the original DMNA-transformed cell lines. These revertant cultures had a decreased saturation density and showed contact inhibition of cell replication. Revertant cells were able to form colonies on glutaraldehyde fixed normal cells (unlike the original lines) and showed a decreased cloning efficiency on feeder layers and in soft agar. Like variants from tumors induced by polyoma virus-transformed cells, the revertant lines showed a higher tumor formation following sc inoculation into adult hamsters than did the original DMNA-transformed lines. Most (82-89%) of the variant colonies from

tumors produced by DMNA-transformed cells exhibited a finite in vitro life span as compared to only 4% of the variant colonies from tumors produced by polyoma-transformed cells. Sixteen to 18% of the variant colonies isolated from DMNA-transformed cell tumors re-reverted to a transformed state as evidenced by re-establishment of a high saturation density and increased cloning efficiency on feeder layers and in soft agar.

137. VIRUS DEOXYRIBONUCLEIC ACID SEQUENCES IN SUBDIPLOID AND SUBTETRAPLOID REVERTANTS OF POLYOMA-TRANSFORMED CELLS.

Shani M, Rabinowitz Z, Sachs L
Weizmann Inst. Sci., Rehovot, Israel
J Virol; 10(3):456-461 1972

Polyoma-transformed hamster cells which had reverted from the properties of transformed cells, while continuing to produce polyoma-specific T antigen and virus-specific RNA, were examined. These variant cells were subdiploid (with modal chromosome numbers of 39-42) or subtetraploid (modal numbers of 73-76). The number of polyoma DNA equivalents in DNA from revertants was determined and compared with that in polyoma-transformed cells (modal chromosome numbers of 44 and 45). Both transformed and revertant cells had an average of eight viral DNA copies/cell. The results indicate that duplication of chromosomes in subtetraploid revertants does not include chromosomes carrying the viral genome. Viral DNA in the revertants was associated with high-molecular wt cell DNA, as in the transformed cells.

138. CHROMOSOMAL CONTROL OF REVERSION IN TRANSFORMED CELLS.

Hitotsumachi S, Rabinowitz Z, Sachs L
Weizmann Inst. Sci., Rehovot, Israel
Nature (Lond); 231(5304):511-514 1971

Direct evidence is presented that the expression (E) or suppression (S) of malignancy of transformed properties depends on the balance between chromosomes with E and S factors. The chromosome groups that contain these types of chromosomes were identified by the use of revertants from transformed cells with different degrees of suppression of transformed properties. Eight variants with a reversion of properties of transformed cells were examined together with normal and transformed cells for their properties in vitro. Reversion in these variants was not associated with a loss of the virus genome. The cells were tested in vivo for their ability to form tumors after their subcutaneous inoculation into adult animals, and in vitro for their saturation density, cloning efficiency in liquid medium and soft agar, and the percentage of colonies at 41 C. The variants were then grouped on the basis of their degree of suppression of transformed properties. The karyotypes of the variants and of normal and transformed cells were studied. From this it was possible to show the number of chromosomes related to the degree of expression and the variant group in which they were present. Transformation in the polyoma transformed cells was associated with an increase in the number of chromosomes with E which established an excess of E over S. Reversion in the subdiploid variants was associated with a specific chromosome loss which resulted in an excess of S over E. This excess was sufficient to suppress the transformed properties, in spite of maintenance of the viral genome in reverted cells. It was suggested that the treatment of cells with agents which change the chromosome balance so as to produce an excess of S may be of value in tumor therapy.

139. CHROMOSOMAL MECHANISM FOR THE INDUCTION OF REVERSION IN TRANSFORMED CELLS.

Block-Shtacher N, Rabinowitz Z, Sachs L
Weizmann Inst. Sci., Rehovot, Israel
Int J Cancer; 9(3):632-640 1972

A clone of hamster embryo cells transformed by polyoma virus was grown in culture at low cell density (103 cells/ 50 mm dish) at 41 C in the absence of a feeder layer to induce reversion of transformation. By six days of culture, the percentage of colonies in the cultured cells decreased from 79 to 33%, implying an increase in revertant cells, or cell variants. The increased frequency of variants was proportional to the increase frequency of aneuploid cells in reverting cultures. In six days of culture the percentage of diploid cells dropped from 78 to 33% and the percentage of aneuploid cells rose from 18 to 62%. Most aneuploid cells had chromosome numbers of 70-87. Changes in chromosome number and frequency of cell variants were not found in transformed cells seeded in culture at 105 cells/dish. The reverted state induced by growth at low cell density was unstable, but could be stabilized by incubation at 24 C. Chromosome numbers and percentage of colonies were not changed by incubation at 24 C. In studies of the number and size of nuclei in revertant cultures, it was found that binucleated cells developed which gave rise to cells with a large nucleus. The latter cells were apparently polyploid, and lost chromosomes become aneuploid variant cells. Stabilization of the revertant state is probably associated with the production of the right type of stable aneuploid cells required for reversion.

140. CHROMOSOMAL CONTROL OF MALIGNANCY IN TUMORS FROM CELLS TRANSFORMED BY POLYOMA VIRUS.

Yamamoto T, Hayashi M, Rabinowitz Z, Sachs L
Dept. Genetics, Weizmann Inst. Sci., Rehovot, Israel
Int J Cancer; 11(3):555-566 1973

The chromosome banding pattern was analyzed in clones of male and female golden hamster cells independently transformed by polyoma virus and tumors derived from these cells to identify the chromosomes with the genes of expression and suppression for malignancy. All the tumors showed either a loss of a piece of chromosome 53 or a gain of a piece or a whole chromosome of 56. An additional 72 chromosome in one line of transformed cells was lost in the tumors derived from this line. The results support the authors' gene balance model of malignancy and indicate that genes of expression of malignancy in these cells were located on chromosome 56 and that genes for suppression of malignancy were located on a piece of chromosome 53 and on chromosome 72. All transformed lines and their tumors showed a loss of a piece of one or both X chromosomes in male and female cells, resp. This loss of a piece of X chromosome was also found in non-malignant revertants from polyoma-transformed cells, so that this change in the X chromosome was not specific for malignant cells.

141. PHENOTYPIC VARIATION AND ITS CONTROL IN POLYOMA-TRANSFORMED BHK21 CELLS.

Wyke J
Dept. Microbiology, Sch. Medicine, Univ. Washington,
Seattle, WA 98105
Exp Cell Res; 66(1):209-223 1972

Dividing cells incorporate the thymidine analogue bromodeoxyuridine (BUdR) into their DNA and become lethally sensitive to irradiation with blue light. Polyoma virus-transformed hamster cells acquire the ability to grow in suspension (in semi-solid media). The BUdR + blue light selection method was applied to various clones of transformed

BHK21 cells growing in suspension to detect variants of transformed cells that will not grow in suspension, since the nondividing cells would survive the treatment. Surviving cells were cloned on solid media, and variants were chosen for further study if they possessed a stable altered solid-surface colony morphology with respect to that of the original transformed clone from which they were derived. The variants reached growth density saturation at one-fifth the level of the parent clones; this limitation was found to depend on the serum concentration of the medium, suggesting that the variants have a higher requirement for serum or a higher sensitivity to medium depletion. Additional evidence suggests that reduced growth in suspension might be associated with polyploidy but that it is not necessarily correlated with morphological variation. The variant cells were slightly larger than the transformed parent cells, and were inhibited or killed by close cell to cell contact. Variant cell cultures tended to segregate colonies of typically transformed morphology and size ('re-transformed' cells). It was found that variant cells were subtetraploid in karyotype in contrast to the essentially diploid-karyotypes of the parent clones; retransformed cells also had karyotypes in the diploid range. Variant cells possessed the polyoma-specific T antigen, indicating that viral genes are still present in these cells. Furthermore, it was demonstrated that the variant morphology was not associated with loss of tumorigenicity (measured by intradermal cheek pouch inoculation), in fact, the tumorigenicity of some variants was enhanced in comparison with the parent clone. Additional evidence suggests that the polyoma-specific transplantation rejection antigen is reduced or modified in the variant cells. Possible mechanisms for the control of transformed and variant phenotypes are discussed. (26 Refs)

142. REVERSION OF VIRUS-TRANSFORMED CELL LINES: HYPERPLOIDY ACCOMPANIES RETENTION OF VIRAL GENES.

Pollack R, Wolman S, Vogel A
New York U. Med. Sch., New York
Nature (Lond); 228(5275):938 1970

Chromosome number and growth control of virus-transformed mouse cell lines and their revertants were examined. The 4 established cell lines (3T3, 3T6, SV3T3, and Py3T3) had similar chromosome modes (73, 76, 74, and 70, resp) although only 3T3 had growth control. Selected variants (F13T6, F1SV3T3, and F1Py3T3) exhibited increased chromosome numbers (195, 118, and a bimodal 60, 120, resp), while the revertants (ReSV3T3 and RePy3T3) had chromosome numbers (72 and 65, resp) close to those of the transformed parent lines. An increase in chromosome number accompanies reversion, and a decrease accompanies back reversion.

143. ISOLATION AND CHARACTERIZATION OF REVERTANT CELL LINES. II. GROWTH CONTROL OF A POLYPLOID REVERTANT LINE DERIVED FROM SV40-TRANSFORMED 3T3 MOUSE CELLS.

Pollack R, Vogel A
Cold Spring Harbor Lab., N.Y.
J Cell Physiol; 82(1):93-106 1973

A polyploid revertant line F1SV101, isolated from an SV40-transformed 3T3 mouse fibroblast culture, was previously shown to have a low maximum cell density in spite of the continued presence in the revertant of SV40-specific DNA, RNA and T-antigen. The mechanism is now examined by which the revertant maintains a low saturation density. The fraction of cells in mitosis was determined by exposure of cells to Velban, trypsinization, fixation, staining and microscopic examination of at least 1000 nuclei. DNA synthesis was followed by incorporation of 32P-phosphate or 3H-thymidine, and determination of fraction of cells synthesizing DNA was

carried out by radioautography of incorporated 3H-thymidine. It was found that at confluence the revertant line F1SV101 and the original fibroblast line 3T3 were both reduced in mitotic index, fraction of cells synthesizing DNA, and rate of DNA synthesis. It appeared that the low saturation density of F1SV101 and 3T3 could not be due to a balance between continued DNA synthesis at the rate found in logarithmically growing cells and cell death, since none of the lines shed more than 2% of the cells per day.

144. ISOLATION AND CHARACTERIZATION OF REVERTANT CELL LINES. III. ISOLATION OF DENSITY-REVERTANTS OF SV40-TRANSFORMED 3T3 CELLS USING COLCHICINE.

Vogel A, Risser R, Pollack R
Cold Spring Harbor Lab., N.Y.
J Cell Physiol; 82(2):181-188 1973

Treatment of the sarcoma virus 40 (SV40) transformed 3T3 cell line, SV101, with 0.5 microg/ml colchicine permits the isolation of polyploid revertant sublines which have lower saturation densities in 10% calf serum than SV101. These low saturation density lines have also reverted to a high serum requirement for growth, and are unable to form colonies in methocel. Normal SV40 has been recovered from these revertants. All colchicine revertants contain more chromosomes than SV101, with modal values varying from 80-90 chromosomes/cell. The DNA content/cell of the revertants is also increased. 3T3 cells are more resistant to colchicine than SV3T3 cells at all cell densities. Colchicine revertants do not display a 3T3-like resistance to colchicine at low density, but do survive colchicine at confluent cell densities, presumably due to their increased contact inhibition.

145. ISOLATION AND CHARACTERIZATION OF REVERTANT CELL LINES. IV. DIRECT SELECTION OF SERUM-REVERTANT SUBLINES OF SV40-TRANSFORMED 3T3 MOUSE CELLS.

Vogel A, Pollack R
Cold Spring Harbor Lab., N.Y.
J Cell Physiol; 82(2):189-198 1973

SV101, the sarcoma virus 40-transformed subline of the mouse fibroblast line 3T3, is both serum- and density-transformed, since it grows in both 1% and 10% calf serum, and grows beyond confluence in 10% calf serum. Negative selection at low cell density in 1% calf serum or in 10% agamma-depleted serum permits direct recovery of serum-revertant sublines of SV101. These sublines are unable to grow in 1% calf serum. Although negative selection at high cell density in 10% calf serum is known to permit recovery of density-revertant sublines of SV101, most density-revertants selected with FUdr or BUdr are not serum-revertant. However, all serum-revertants isolated so far are density-revertant as well. As has been reported for density revertants, serum revertants contain more DNA and more chromosomes/cell than their SV101 parent.

146. ISOLATION AND CHARACTERIZATION OF REVERTANT CELL LINES. VI. SUSCEPTIBILITY OF REVERTANTS TO RETRANSFORMATION BY SIMIAN VIRUS 40 AND MURINE SARCOMA VIRUS.

Vogel A, Pollack R
Cold Spring Harbor Lab., N. Y.
J Virol; 14(6):1404-1410 1974

The equilibrium of the viral and cellular genomes in simian virus 40 (SV40)-transformed 3T3 cells was investigated by reinfection of the cells with either SV40 or Kirsten sarcoma virus (SV)(R-MLV). Serum-sensitive and density-sensitive revertant cells were each infected with each virus and the resulting cell characteristics were studied. Neither of the revertant groups were retransformable by SV40, but both

groups were transformed by the Kirsten SV, the cells becoming round and spindly. The density-sensitive group became able to grow in high cell densities and to form colonies in methyl cellulose suspension; the serum sensitive group, in contrast, did not regain the ability to grow in low serum concentrations. No reduction in chromosome number accompanied the transformation. It is concluded that genome equilibrium in SV40-transformed 3T3 cells is not at the expense of the cell's phenotypic expression, and that the two types of virus do not have the same effect on the cell.

147. ISOLATION AND CHARACTERIZATION OF REVERTANT CELL LINES. VII. DNA SYNTHESIS AND MITOTIC RATE OF SERUM-SENSITIVE REVERTANTS IN NON-PERMISSIVE GROWTH CONDITIONS.

Vogel A, Pollack R

Cold Spring Harbor Lab., N. Y.

J Cell Physiol; 85(1):151-162 1974

The ability of BALB/c and Swiss mouse 3T3 cells, simian virus 40 (SV40)- and murine sarcoma virus (MSV)-transformed 3T3 cells, and serum-sensitive revertants of the transformed cells to synthesize DNA and enter mitosis were studied under conditions of confluence and low serum concentrations. The untransformed cells were able to maintain a constant population number under these nonpermissive growth conditions by various means: cessation of DNA synthesis, DNA synthesis coupled with failure to enter mitosis, and slow traverse of the cell cycle coupled with cell shedding. Growth control of the revertant of an MSV-transformed BALB/3T3 cell line most closely resembled that of the nontransformed 3T3 lines. This line did not grow in 1% serum and did not synthesize DNA under either nonpermissive conditions. The serum-sensitive revertants of the SV40-transformed 3T3 cells were also unable to grow in 1% serum and did not grow beyond confluence in 10% serum, but these cells differed from the nontransformed 3T3 lines in the manner in which this growth arrest was accomplished. In 1% serum the revertants synthesized DNA but did not enter mitosis, while at confluence in 10% serum, they slowly traversed the cell cycle, with dividing cells replacing cells which were shed into the medium. The results suggest that a major effect of transformation is to render 3T3 cells independent of any serum requirement for the initiation of cellular DNA synthesis.

148. STIMULATION OF CELL GROWTH IN VITRO BY SERUM WITH AND WITHOUT GROWTH FACTOR. RELATION TO CONTACT INHIBITION AND VIRAL TRANSFORMATION.

Jainchill JL, Todaro GJ

Columbia-Presbyterian Med. Ctr., New York, N.Y.

Exp Cell Res; 59(1):137-146 1970

Comparisons were made of the growth rates of sparse and confluent cultures of 3T3 and BALB/3T3 mouse cell lines; SV40-transformed cells derived from 3T3 and BALB/3T3 which had lost contact inhibition of cell division; SV-F1-101, a variant derived from a cloned line of SV40-transformed 3T3 which had a relatively low saturation density; and BALB/3T3 cells transformed by murine sarcoma virus (MSV) which had lost contact inhibition. In general, cell lines with a low saturation density were unable to grow in a medium deficient in serum growth factor (sgf) while transformed cells grew well under these conditions. The exception was SV-F1-101 in which low saturation density appeared to be dissociated from a high requirement for sgf. Although SV-F1-101 required little sgf, it maintained a low cell density despite a high division rate. The low cell density was apparently the result of an equilibrium between proliferation and detachment. BALB/3T3 cells transformed by MSV which had lost contact inhibition were able to grow on medium deficient in sgf while 'revertant' subclones which were contact inhibited, and no longer shed

MSV, were not. Murine leukemia virus can chronically infect BALB/3T3 but, unlike MSV, it did not change either the saturation density or ability to grow in growth-deficient medium. The continued presence of SV40 and MSV genetic material in the cells was apparently associated with a pronounced decrease in the requirement for sgf.

149. DIRECT ISOLATION AND CHARACTERIZATION OF 'FLAT' SV40-TRANSFORMED CELLS.

Scher CD, Nelson-Rees WA

Natl. Cancer Inst., Bethesda, Md.

Nature (Lond); 233(43):263-265 1971

SV40-transformed BALB/c-3T3 mouse embryo lines were grown in serum factor-free medium and subsequently were cloned. The transformed cells thus derived had disoriented growth patterns, possessed T antigen and exhibited a spectrum of saturation densities. Fifteen percent of the clones isolated were 'flat' revertants exhibiting relatively low saturation densities which were approximately the same as those of the nontransformed 3T3 cells. Five out of six 'flat' lines synthesized DNA at confluency with 50 to 70% of the cells incorporating 3H-thymidine. Only one 'flat' transformant line behaved like normal 3T3 cells at confluency, with only about 1% of the cells incorporating 3H-thymidine. This line was still able to synthesize DNA, since incorporation of 3H-thymidine could be induced by adding fresh serum to the medium. The only virus altered growth property of these cells was the ability of sparse cells to grow in serum factor-free medium. The saturation density of 'flat' transformants that continue to synthesize DNA while confluent is determined by an equilibrium between cell division and cell death; 48% of the cells in a confluent layer of one 'flat' transformant line were observed to undergo mitosis, although the total number of cells in the culture remained the same. Previous observation had indicated that 'flat' revertants of SV40 and polyoma-transformed 3T3 cells undergo a shift in chromosome number from a hypotetraploid to a hypertetraploid state, with a concomitant increase in the number of metacentrics. However, in this study, three out of four 'flat' transformants which continued to synthesize DNA at confluency remained hypotetraploid. Only one line studied showed more than 15% of the cells with one or more metacentric chromosomes. It is thus evident that hypertetraploidy is not a prerequisite for 'flat' transformants retaining the ability to synthesize DNA at confluency. It is also evident that low saturation density is not in itself a criterion for density dependent inhibition of cell division.

150. CONTACT-INHIBITED REVERTANT CELL LINES ISOLATED FROM SV40-TRANSFORMED CELLS: I. BIOLOGIC, VIROLOGIC AND CHEMICAL PROPERTIES.

Culp LA, Grimes WJ, Black PH

Harvard Med. Sch., Boston, Mass.

J Cell Biol; 50:682-690 1971

SV40-transformed mouse 3T3 cells (SV-3T3) were exposed to 5-fluoro-2'-deoxyuridine to isolate contact-inhibited 'revertant' cell lines. Two revertant clones were established; revertant cells resembled 3T3 cells morphologically in that both 3T3 and revertant lines contained cells which were large, pleomorphic and polygonal or epithelioid in morphology. Many multi-nucleated giant cells were seen in revertant cultures; giant cells were less common in SV-3T3 cells. SV40 T antigen was detected by fluorescent antibody tests in almost all nuclei of revertant cells, and SV40 could be rescued from revertants by fusion with permissive monkey cells. Transformation frequencies in 3T3 cells exposed to SV40 from revertant cell lines were similar to those in 3T3 cells exposed to wild-type SV40. Revertant cells contained more sialic acid than SV-3T3 cells; the 2 revertant cell lines

contained 4.4 and 4.8 microg sialic acid/mg protein, resp, whereas SV-3T3 cells contained 3.0 microg sialic acid/mg protein. An inverse correlation was found between the saturation density of cells and their sialic acid content. Collagen content was similar in revertant and SV-3T3 cells. Contact inhibition of revertant cells may be related to sialic acid content of the cell plasma membrane.

151. CONTACT-INHIBITED REVERTANT CELL LINES ISOLATED FROM SV40-TRANSFORMED CELLS: II. ULTRASTRUCTURAL STUDY.

McNutt NS, Culp LA, Black PH
Harvard Med. Sch., Boston, Mass.
J Cell Biol; 50:691-708 1971

Contact-inhibited revertant mouse cells, normal 3T3 cells and SV40-transformed 3T3 cells were observed under the electron microscope; confluent and subconfluent cultures of normal and virus-transformed 3T3 cells were studied. Revertant cells were usually mononucleate but tended to form single- and multinucleated giant cells. Nuclear pleomorphism in revertant cells was striking. Nuclei of revertant cells showed more variation in morphology than did nuclei in normal 3T3 cells; normal cell nuclei were smaller than nuclei of revertant cells. Nuclei of transformed 3T3 cells were smaller than either revertant cell nuclei or normal cell nuclei. Deep infoldings of both membranes in the nuclear envelope of the nucleoplasm were more common in confluent virus-transformed 3T3 cells than in confluent normal 3T3 cultures or revertant cell cultures. The endoplasmic reticulum of normal 3T3 cells resembled that in revertant cells in confluent cultures. Endoplasmic reticulum in transformed cells was seldom organized into a branching system of cisternae and tubules; this arrangement was more frequently seen in revertant and in normal cells. Ectoplasm of both normal and revertant cells showed a prominent system of filaments 60-80 or 100 angstrom in diameter. Microtubules 250 angstrom in diameter were also common in the ectoplasm of normal and revertant cells. Virus-transformed cells had a relatively poorly developed system of ectoplasmic filaments. The abundance of these filaments correlated with contact inhibition of movement and growth of cells; fewer bundles of filaments were seen in growing cells than in contact-inhibited cells.

152. CONTACT-INHIBITED REVERTANT CELL LINES ISOLATED FROM SIMIAN VIRUS 40-TRANSFORMED CELLS. III. CONCANAVALIN A-SELECTED REVERTANT CELLS.

Culp LA, Black PH
Harvard Med. Sch., Cambridge, MA
J Virol; 9(4):611-620 1972

Four revertant clones were isolated from SV40-transformed BALB/c 3T3 (SVT2) cell cultures grown in the presence of concanavalin A (con A). The revertant cells resembled normal BALB/c 3T3 cells in that they maintained a flat morphology in either subconfluent or confluent cultures, formed monolayers, and grew to similar saturation densities. The SV40-specific T antigen was detected in more than 90% of the nuclei in SVT2 cultures and in all four revertant clones, by indirect immunofluorescence staining. All four revertant clones yielded infectious virus after fusion with a permissive monkey kidney cell line mediated by inactivated Sendai virus. Three of the revertant clones had a high sialic acid content similar to that of BALB/c 3T3 cells and not to that of SVT2 cells. The consistently low sialic acid content in the fourth revertant clone was associated with a very high propensity of this clone to 'revert' to spindle-shaped cells which formed multilayers. Confluent or subconfluent SVT2 and revertant clones produced about twice as much collagen as did BALB/c 3T3 cells. The revertant clones contained approximately twice the number of chromosomes (90-99) as did the parental SVT2

cells; however, the qualitative cytogenic characteristics of the SVT2 and the revertant cells were similar.

153. CONTACT-INHIBITED REVERTANT CELL LINES ISOLATED FROM SV40-TRANSFORMED CELLS. V. CONTACT INHIBITION OF SUGAR TRANSPORT.

Schultz AR, Culp LA
Case Western Reserve U., Sch. Med., Cleveland, OH
Exp Cell Res; 81:95-103 1973

The transport of 2-deoxyglucose in BALB/c 3T3 cells, Simian virus 40-transformed BALB/c 3T3 (SVT2) cells, and concanavalin A-selected revertant cells of SVT2 was measured. Sparsely-seeded BALB/c 3T3 cells transport the sugar at about 1/4, and sparsely-seeded revertant cells at 3/4, the rate of SVT2 cells. BALB/c 3T3 cells undergo a dramatic drop in sugar uptake at confluency, transporting sugar at about 1/10 the rate of subconfluent cells. Revertant cells (contact-inhibited variants of transformed cells) are similar in this respect, but the drop is only 5-fold. SVT2 cells show no such change in uptake over wide cell densities. Subconfluent BALB/c 3T3, SVT2, and revertant cells have similar K_m and V_{max} values for 2-deoxyglucose transport; however, confluent 3T3 and confluent revertant cells show a large increase in K_m and a 5-fold decrease in V_{max} as compared with their subconfluent counterparts or SVT2 cells - indications of a decreased number of transport sites and a decreased affinity of these sites for sugar when these cells make intimate contacts with each other. These data indicate that extensive changes in the architecture of the cell surface occur when contact-inhibited cells are in close apposition with each other, regardless of the persistence of partially expressed SV40 genetic information. The data also provide support for the hypotheses that transport sites in confluent, contact-inhibited cells are covered by an additional membrane component or changed configurationally. The change in K_m in association with the change in V_{max} suggests a steric change in the membrane of these cells.

154. GENETIC STABILITY OF THE SARCOMA VIRUSES IN MURINE AND AVIAN SARCOMA VIRUS-TRANSFORMED NONPRODUCER CELLS.

Stephenson JR, Scolnick EM, Aaronson SA
Natl. Cancer Inst., Bethesda, MD
Int J Cancer; 9(3):577-583 1972

Clonal lines were obtained by a microtiter technique from cultures of normal rat kidney (NRK) and BALB/3T3 cells transformed by Kirsten murine sarcoma virus (Ki-MSV) or by Schmidt-Ruppin Rous sarcoma virus (SR-RSV). These clones, in which the virus did not productively replicate, were studied for the occurrence of spontaneous or chemical (BrdU or mitomycin-C)-induced morphologic reversion. Although no spontaneous revertants were detected, four morphologically revertant clones were obtained from over 10,000 transformed clones studied. One (K-NRK-R), which was isolated from a BrdU-treated Ki-MSV-transformed NRK culture, was further characterized. The K-NRK-R clone was similar to NRK control cells in saturation density and its ability to grow on monolayers. Infectious virus could be rescued from K-NRK-R cells by superinfection with murine leukemia virus, suggesting that the revertant line arose as a result of a mutation of a cellular gene involved in expression of the transformed state, and not from loss or mutation of the viral genome.

155. CHARACTERIZATION OF MORPHOLOGIC REVERTANTS OF MURINE AND AVIAN SARCOMA VIRUS-TRANSFORMED CELLS.

Stephenson JR, Reynolds RK, Aaronson SA
Natl. Cancer Inst., Bethesda, MD
J Virol; 11(2):218-222 1973

Growth characteristics, tumorigenicity, and ¹⁴C-glucose uptake were examined in morphological revertant cells isolated from mouse cells transformed by Kirsten murine sarcoma virus (KiMSV) or Schmidt-Ruppin Rous sarcoma virus. Saturation densities of revertant lines were well below those of parent transformed cell lines, and were similar to those of nontransformed parent mouse cells. Colony-forming efficiency (CFE) of revertants, like that of normal, nontransformed parent cells, was markedly lower than the CFE of transformed parent lines. Glucose uptake had reverted to normal in revertant cells and the malignant potential of revertants was reduced relative to that of transformed parent cells. Revertant lines and transformed cells contained similar concentrations of avian group-specific antigen. Revertants from KiMSV-transformed parent lines showed reduced susceptibility to transformation by KiMSV.

156. REVERSION OF MURINE SARCOMA VIRUS TRANSFORMED MOUSE CELLS: VARIANTS WITHOUT A RESCUABLE SARCOMA VIRUS.

Fischinger PJ, Nomura S, Peebles PT, Haapala DK, Bassin RH

Natl. Cancer Inst., Bethesda, MD
Science; 176(4038):1033-1035 1972

Extended cultivation of single colonies of murine sarcoma virus (MSV)-transformed mouse 3T3 cells (S+L-), which were negative for murine leukemia virus (MuLV) and which yielded sarcoma virus after super-infection with MuLV, resulted in the formation of spontaneous flat revertants from which MSV could no longer be rescued. The revertants were able to support MuLV growth and showed an enhanced sensitivity to MSV superinfection. Like normal cells, the flat revertants did not release RNA-dependent DNA polymerase activity into the supernatant fluid, nor did they have any detectable C-type particles. Because revertants could be obtained with high frequency from progeny of single transformed cells, each cell that contained the MSV genome seemed to have the capacity to suppress or eliminate an RNA tumor virus native to its species of origin.

157. REVERTANTS OF MOUSE CELLS TRANSFORMED BY MURINE SARCOMA VIRUS. II. FLAT VARIANTS INDUCED BY FLUORODEOXYURIDINE AND COLCEMID.

Nomura S, Fischinger PJ, Mattern CF, Gerwin BI, Dunn KJ

Natl. Cancer Inst., Bethesda, MD
Virology; 56(1):152-163 1973

Mouse sarcoma virus-transformed 3T3FL cells (S+L-cells) produced spontaneously and at variable rates flat variants with some properties of nontransformed cells. The frequency of occurrence of such variant cells increased after treatment of S+L-cells with fluorodeoxyuridine (FdUrd) or Colcemid. In one S+L- subline (3-360), the spontaneous flat variants (S) occurred at the rate of about 1 in 80 clonal colonies, and, after treatment with Colcemid or FdUrd, flat variants were observed in 1 of 20 and 30 colonies, respectively. In another S+L- subline (3-321), S variants normally occurred less than 1 in 1000 colonies, and the same treatments increased the frequency to approximately 1 in 70 and 55 colonies, respectively. Both the FdUrd and Colcemid-induced flat variants (F and C) resembled 3T3FL cells morphologically, grew to low saturation densities, and exhibited cloning

efficiencies in soft agar which were 10-1 except for one S subline, they contained murine leukemia group-specific antigen(s) without demonstrable virus production and reverse transcriptase activity. Murine sarcoma virus (MSV) was no longer rescuable by superinfection with murine leukemia virus (MuLV) or by cell fusion with 3T3FL, BALB/3T3, or normal rat kidney cells. All flat variant cells were susceptible to MSV and MuLV infection, and some degree of enhancement of sensitivity to MSV and MuLV infection was observed in most variant cultures. All flat variant sublines possessed the property of agglutinability by concanavalin A as high as that of S+L- cells. Some flat variant clones spontaneously underwent retransformation during extended cultivation. In contrast to previously described S+L- cell revertants, which spontaneously retransformed morphologically without a rescuable MSV genome, in the present experiments retransformation with a rescuable MSV genome was observed in one of four S sublines and two of five F sublines, but in none of four C sublines. Furthermore, one of five F sublines gave rise to a clone which was flat but which otherwise resembled S+L- cells. In the S and C variants, the loss of expression of transformation was associated with an increase in chromosome number. However, the chromosome number of F variants was similar to or slightly less than that of parental S+L- cells. These studies demonstrated that the reversion of S+L- cells to flat variants occurred by different mechanisms apparently involving either the viral or cellular genes or both, and that some flat variant sublines had retained at least one complete MSV genome in nondetectable form.

158. REVERTANTS OF MOUSE CELLS TRANSFORMED BY MURINE SARCOMA VIRUS: FLAT VARIANTS WITHOUT A RESCUABLE SARCOMA VIRUS FROM A CLONE OF BALB/3T3 TRANSFORMED BY KIRSTEN MSV.

Nomura S, Dunn KJ, Mattern CF, Hartley JW, Fischinger PJ

Natl. Cancer Inst., Bethesda, MD
J Gen Virol; 25(2):207-218 1974

A clonal subline of Kirsten murine sarcoma virus (Ki-MSV)-transformed nonproducer BALB/3T3 (Ki-BALB) cells spontaneously produced flat variants with some properties of nontransformed cells at frequencies of 0.01-0.001. The variants were epithelioid, contact-inhibited, grew to low saturation density, and exhibited variable cloning efficiencies in soft agar. They demonstrated no murine leukemia group-specific antigen(s), no reverse transcriptase activity, and no infectious virus but were agglutinable by concanavalin A. Murine leukemia virus (MuLV) was induced after 5-fluorodeoxyuridine (IUDR, 20 microg/ml) treatment from both the flat variants and from the parental Ki-BALB cells. However, Ki-MSV could not be rescued from these flat variants by superinfection with MuLV nor induced by IUDR treatment. The state of reversion was stable, revealing no back-transformation during 30-40 subcultures. The flat variants produced no tumors in syngeneic mice inoculated sc with 104, 105, or 106 cells, whereas the same numbers of Ki-BALB cells produced tumors in 8 of 10, 5 of 5, and 6 of 6 animals, resp. All flat variants were susceptible to MSV and MuLV infection. The loss of the transformed phenotype in spontaneous flat variants was associated with a decrease in chromosome number to a level similar to BALB/3T3 cells. These observations suggest that these revertants may have lost some or all of the Ki-MSV genome.

159. MUTANTS OF NONPRODUCER CELL LINES TRANSFORMED BY MURINE SARCOMA VIRUSES.

Hatanaka M, Klein R, Toni R, Walker J, Gilden R
Flow Lab., Inc., Rockville, MD
J Exp Med; 138(2):356-363 1973

A variety of cell mutants were obtained by a single 5'-bromodeoxyuridine treatment of a nonproducer cell line transformed by the Kirsten strain of murine sarcoma virus (Ki-MSV). Isolation procedures of these cell mutants are described. The cell mutants obtained were classified by tumorigenic potential and shedding of Type C virus particles into four groups: 1) tumorigenic, without particles; 2) tumorigenic, with Type C particles; 3) nontumorigenic, without particles; and 4) nontumorigenic, with Type C particles. The tumorigenic cell lines showed variability in morphology with both flat and typical transformed cell lines of equal transplantability. The oncogenic properties remained constant through multiple passages over a six-month period.

160. MUTANTS OF NONPRODUCER CELL LINES TRANSFORMED BY MURINE SARCOMA VIRUS. II. RELATIONSHIP OF TUMORIGENICITY TO PRESENCE OF VIRAL MARKERS AND RESCUABLE SARCOMA GENOME.

Hatanaka M, Klein R, Long CW, Gilden R
Flow Labs., Inc., Rockville, MD
J Exp Med; 138(2):364-372 1973

Tumorigenic and nontumorigenic cell mutants induced by a single 5'-bromodeoxyuridine (BrdU) treatment of a nonproducer (NP) tumorigenic cell line were isolated by cloning and characterized. Among the cloned derivatives were examples of virus-free and sarcoma virus-producing cell lines. Oncogenicity did not correlate with production of virus or ease of rescue of the sarcoma genome. All lines, including nononcogenic derivatives, retained the sarcoma genome. Phenotypic reversion of some cell mutants was observed after in vivo inoculation or long term in vitro cultivation. The M-50T cell line, obtained from a tumor induced by M-50 mutant cells, had a sarcoma genome rescueable by direct superinfection; this was only achieved with parental M-50 cells by a cell fusion rescue technique. The M-43-2T cell, obtained from a single small static tumor induced by otherwise nononcogenic M-43-2 mutant cells, shed sarcoma virus and became tumorigenic. M-58-4-48 became tumorigenic after passage of 48 of the M-58-4 mutant line, which was originally nontumorigenic. These observations of phenotypic reversion demonstrate that the presence of the sarcoma gene in cells is an essential but not sufficient condition of tumorigenesis.

161. MUTANTS OF NONPRODUCER CELL LINES TRANSFORMED BY MURINE SARCOMA VIRUS. III. DETECTION AND CHARACTERIZATION OF RNA SPECIFIC FOR HELPER AND SARCOMA VIRUSES.

Tsuchida N, Shih M, Gilden RV, Hatanaka M
Flow Labs., Inc., Rockville, MD
J Exp Med; 140(1):218-224 1974

BALB/3T3 cells transformed by the Kirsten sarcoma virus (Ki-SV) (non-virus producer BALB/3T3 cells) and mutant cell lines derived therefrom, following treatment with 5'-bromodeoxyuridine (BrdU) were analyzed for the expression of virus-specific RNA using single-stranded DNA transcripts of Rauscher leukemia virus (RLV), a virus activated in one of the cell lines (58-2T), and Ki-SV-specific DNA transcript (after removal of all sequences crossreactive with RLV DNA). The Rauscher virus DNA detected multiple copies of viral RNA in virus-producing cells (about 2.5×10^3 /cell) whether infected with RLV or activated to produce virus with BrdU. Nonproducer (NP) cells and normal BALB cells showed small numbers of RNA genomes (70-250/cell)

and only partial saturation of the transcripts. The intracellular RNA sedimented at 35S (main peak) with a variable minor peak at 20S with the exception of one mutant cell, M-43-2, which showed a main peak at 26-27S. The 58-2T transcript reacted preferentially in NP cells and their derivatives with biphasic kinetics suggesting the possibility of sequences specific for the original transforming virus. The size of the Ki-SV specific sequences was 30S in the mutant cells whether or not complete virus was being produced and independently of in vivo transplantability.

162. SELECTION OF REVERTANTS OF KIRSTEN SARCOMA VIRUS TRANSFORMED NONPRODUCER BALB/3T3 CELLS.

Ozanne B, Vogel A
Cold Spring Harbor Lab., NY
J Virol; 14(2):239-248 1974

Revertants of Kirsten sarcoma virus-transformed nonproducer BALB/3T3 cells (KA31 cells) were isolated after exposure to 5-fluorodeoxyuridine at high cell density or when suspended in methylcellulose. Revertants were also isolated by treating KA31 cells with concanavalin A, which was much more toxic for transformed cells than for normal cells. The revertants resembled BALB/3T3 cells in their morphology and growth characteristics: they had a low saturation density, failed to grow in 1% calf serum or when suspended in methylcellulose, and ceased to synthesize DNA after reaching their saturation density. Infection by murine leukemia virus rescued Kirsten sarcoma virus only from the concanavalin-A-selected variants, although all of the revertants were susceptible to infection by leukemia virus. The concanavalin A revertants also became transformed after infection with murine leukemia virus. All of the revertants could be transformed by Kirsten sarcoma virus but not by simian virus 40.

163. REVERSION AND INDUCTION OF ROUS SARCOMA VIRUS EXPRESSION IN VIRUS-TRANSFORMED BABY HAMSTER KIDNEY CELLS.

Boettiger D
Imp. Cancer Res. Fund Lab., London, England
Virology; 62(2):522-529 1974

Revertants from a clone of baby hamster kidney cells transformed by Schmidt-Ruppin virus were isolated by morphological criteria. In contrast to revertants isolated from other virus-transformed mammalian cells, revertant subclones occurred at high frequency, and the degree of reversion varied from subclone to subclone. A range of phenotypes from transformed to normal was observed. All revertant subclones retained the viral genome. Virus could be rescued by Sendai virus-mediated cell fusion with susceptible chicken cells; however, the frequency of virus rescue from the revertant subclones was much lower than the frequency of virus rescue from transformed subclones. Treatment of revertant subclones with 5-bromodeoxyuridine (less than 100 microg/ml) before cell fusion led to a 10-fold increase in virus rescue frequency. It is concluded that reversion is caused by modification in the expression of viral or cellular genes required to maintain the transformed phenotype.

164. REVERTANTS OF HUMAN CELLS TRANSFORMED BY MURINE SARCOMA VIRUS.

Cho HY, Cutchins EC, Rhim JS, Huebner RJ
Microbiological Associates, Inc., Bethesda, MD 20014
Science; 194(4268):951-953 1976

Revertants of nonproducer human osteosarcoma (NP/KHOS) cells induced by Kirsten murine sarcoma virus (Ki-MSV) were isolated and characterized after incubation at 40.5 C overnight and subcloning at 36 C. During incubation, transformed cells were removed and the remaining attached

cells were cloned by the cylinder technique. Two clones (240S and 312H), which appeared stable after 10 mo, exhibited growth properties similar to those of the nontransformed, parent human osteosarcoma (HOS) cells and did not release RNA-dependent DNA polymerase activity. These morphologic variants were nontumorigenic in nude mice. The MSV genome could not be rescued by superinfecting cells with helper type C viruses. The revertants showed a two- to fourfold increase in sensitivity to Ki-MSV compared to HOS and also supported murine leukemia virus growth. Thus, the revertants were from human cells transformed by an oncogenic RNA virus; they showed no increased chromosome numbers when they were compared to their progenitor cells. (16 refs)

165. CLONAL VARIABILITY OF ADENOVIRUS-TRANSFORMED CELLS.

Weber J

Central U. Hosp., Sherbrooke, Quebec, Canada
J Cell Sci; 13(2):421-427 1973

Cells transformed by DNA oncogenic viruses acquire characteristic properties which have been considered criteria for transformation. SV40- and polyoma virus-transformed cells are capable of selective reversion for several of these properties. Until now, reversion of transformed cell properties has not been reported in the adenovirus system. The stability of several transformed cell properties was examined in a line (HTC) of Ad. 12-transformed (in vivo) cells after several hundred generations of in vitro passage. From a large number of clones isolated by the soft-agar suspension technique, 7 were selected for detailed examination. The clones, as the parental cells, were free of infectious virus and type-specific antigen, but contained intranuclear Ad. 12 T antigen. Cell growth profiles with 0, 1 and 10% serum in the culture medium revealed that while 4 clones retained the parental characteristic of growth in the absence of serum, 3 lost this ability and grew only in serum concentrations greater than 1%. Agglutination profiles with 4-fold dilutions of concanavalin A (Con A) and wheat germ agglutinin (WGA) directly or after trypsin treatment revealed 2 clones which failed to agglutinate with Con A; trypsin treatment reversed this effect. Each clone agglutinated with WGA directly, though one clone showed reduced agglutination. On the basis of superinfection with Ad. 2 the clones could be grouped into low, intermediate and high yielders with 10-fold intervals. All clones were transplantable into random-bred adult Syrian hamsters. There was no marked correlation between the reversion or modification of individual transformed cell properties among the clones. The phenotypic stability of these transformed cell properties was confirmed by re-cloning the tumors induced by one of the revertant clones. It was concluded that some of these properties are not essential for the maintenance of oncogenic potential.

B. Conditional Expression of Transformed Cell Properties

166. MUTATION CAUSING TEMPERATURE-SENSITIVE EXPRESSION OF CELL TRANSFORMATION BY A TUMOR VIRUS (MEETING ABSTRACT).

Renger HC, Basilico C

New York U. Sch. Med., N.Y.

Proc Natl Acad Sci USA; 69(1):109-114 1972

Several SV-40 transformed mouse 3T3 cell lines were isolated and characterized according to temperature sensitivity and in vitro growth characteristics. All transformant characteristics were expressed at 32 C but not at 39 C. Transformed lines grew at 32 C but not at 39 C as multilayers,

reached higher saturation densities, had higher cloning efficiencies and grew faster in gamma-globulin-free medium than did transformants at 39 C. T-antigen synthesis occurred in all transformed lines at either temperature. Confluent transformed cells continued to incorporate ³H-thymidine at 32 C but not at 39 C. Inhibition of DNA synthesis at the higher temperature could be reversed by transferring the cultures to the lower temperature. No significant change in chromosome number was noted in the temperature-sensitive transformants. Viruses rescued from all infected lines did not differ in growth characteristics or in transforming ability. It thus appeared that the mutants described were host-cell rather than virus mutants.

167. TEMPERATURE-SENSITIVE SIMIAN VIRUS 40-TRANSFORMED CELLS: PHENOMENA ACCOMPANYING TRANSITION FROM THE TRANSFORMED TO THE 'NORMAL' STATE.

Renger HC, Basilico C

New York U. Sch. Med., NY

J Virol; 11(5):702-708 1973

Temperature-sensitive simian virus (SV40)-transformed 3T3 cells (tsSV3T3), which express the transformed phenotype when growing at 32 C but not at 39 C, were used to study changes in growth behavior during shift-up or shift-down experiments. In cultures of tsSV3T3 cells which had reached or were beyond monolayer density at 32 C, DNA synthesis reached very low levels within 24 to 48 hr after shift-up. When cells which had been allowed to grow to high densities at 32 C were shifted to 39 C, not only cell growth stopped, but within two to three days the cultures shed a large number of cells into the medium. These cells were nonviable, and shedding stopped only when the number of cells attached had been reduced to that characteristic of the saturation density of 39 C. The remaining attached cells were viable and after the shift to 32 C were again able to grow from the monolayer to high cell densities. This behavior is compared with that of normal 3T3 and wild-type SV3T3 cells under different conditions. New tsSV3T3 lines have been isolated, using cells which had been infected with non-mutagenized wild-type SV40. This further demonstrates that the temperature sensitivity of these lines is due to a cellular rather than a viral mutation. Available data suggests that the temperature sensitivity could be due to changes in the surface or serum requirements. The mechanism of cell death which accompanies this phenomenon is still obscure. The data shows, however, that the host mutation carried by the tsSV3T3 cells affects a number of parameters of malignant transformation, and possibly all of them.

168. RETRANSFORMATION OF TS SV40 TRANSFORMANTS BY MURINE SARCOMA VIRUS AT NON-PERMISSIVE TEMPERATURE.

Renger HC

Dept. Pathology, New York Univ. Medical Center, New York City, NY 10016

Nature; 240(96):19-21 1972

The temperature-sensitive, Simian virus (SV40)-transformed mouse fibroblast cell line tsSV3T3 expresses the transformed phenotype at 32 C but not at 39 C. Several facts suggest that the temperature-sensitive phenotype is the result of a cellular rather than a viral mutation: at 39 C, viral-specific T antigen can be demonstrated and viral messenger RNA (mRNA) found by hybridization experiments (indicating that viral mRNA is read), fusion-rescue experiments yield wild-type SV40 particles, and tsSV3T3 cannot be retransformed at 39 C by high multiplicity SV40. However, tsSV3T3 cells have not lost the ability to express a transformed phenotype at 39 C, as they were successfully retransformed at the non-permissive temperature by murine sarcoma virus (MSV) at the same efficiency as normal 3T3 cells were transformed by MSV; the

titration pattern showed a one-hit response in both cases. Similar titers of MSV were released into the medium in 24-48 hr at 39 C by both MSV-transformed tsSV3T3 and MSV-transformed 3T3 cells. Whereas tsSV3T3 were agglutinated by wheat germ agglutinin (WGA) only at 32 C, MSV-transformed tsSV3T3 cells were readily agglutinated at 39 C by concanavalin A (Con A), and this latter event was inhibited by Con A specific hapten. WGA- and Con-A-mediated agglutination are specific indicators of the transformed state. Two alternative hypotheses are proposed and discussed to explain the results. (15 Refs)

169. SV40-TRANSFORMED CELLS WITH TEMPERATURE-DEPENDENT SERUM REQUIREMENTS.

Toniolo D, Basilico C
New York Univ. Sch. Med., NY
Cell (Camb); 4(3):255-262 1975

3T3 cells subconfluent at 32 C were infected with 5 plaque-forming units (PFU)/cell of Simian virus 40 (SV40) that had been mutagenized by 20 ug/ml N-methyl-N'-nitro-nitrosoguanidine to isolate and characterize a temperature-sensitive clone (ts23A). After 24 hr at 32 C in 10% serum, cells were diluted 20-fold, kept for 2-3 d in 10% serum at 32 C, incubated in 10% gammaglobulin-free serum, then incubated in 1% serum. After two d, cells were incubated at 39 C for two d; 5-fluorodeoxyuridine was then added at 30 ug/ml. Cells were washed and retreated twice, then transferred to 32 C. Ts23A cells were unable to form colonies in 1% serum at 39 C (less than 0.01% of plated cells yielded colonies), but were able to form colonies in 10% serum (3.5% yielded colonies). T-antigen, determined by fluorescent antibody staining, was present in ts23A and SV40-transformed 3T3 cells. Ts23A cells were able to form colonies on 3T3 monolayers, as were SV40-transformed 3T3 cells (ratio of colony yield per cell plated on 3T3/colony yield on plates: ts23A, 32 C, 0.96; 39 C, 1.26; SV40-transformed 3T3, 32 C, 0.87; 39 C, 1.18). Ts23A cells were unable to grow in agar suspension. As determined by ability to form plaques, virus rescued from ts23A cells was wild-type (ratio of PFU at 39 C/PFU at 32 C to wild type SV40, 0.73; ts23A virus, 1.89). Unlike 3T3 cells, ts23A cells continued to synthesize DNA after 72 hr at 39 C in 1% serum (frequency of DNA-synthesizing cells as determined by autoradiography with ³H-thymidine pulsing for one hour: 3T3 cells, 2.1%; ts23A cells 13.9%). Ts23A cells had a 26% survival after two d in 1% serum at 39 C. Ts23A cells appear to represent transformed cell variants in which the ability to utilize or take up serum factors has become temperature-dependent.

170. TEMPERATURE-SENSITIVE MUTANTS OF CHEMICALLY TRANSFORMED EPITHELIAL CELLS (MEETING ABSTRACT).

Yamaguchi N, Weinstein IB
Columbia Univ. Coll. Physicians Surg., New York, NY
Proc Natl Acad Sci USA; 72(1):214-218 1975

The first isolation of temperature-sensitive (TS) mutants of epithelial cells transformed with chemical carcinogens was studied. The TS mutant, W-8, was obtained from K-16 normal epithelial rat liver cells exposed to 0.5 microg/ml N-acetoxy-2-acetylaminofluorene. When 105 or 106 W-8 cells were injected sc into newborn Sprague-Dawley rats, 1-2-cm locally invasive tumors composed of epitheloid cells appeared within 3-4 wk. The wild type W-8 clone was obtained by isolating a colony at 41C in 1.2% methyl cellulose. Like the wild type W-8, the TS mutants readily grew in agar suspension at 36 C, but (in contrast to the wild type) they did not grow at 40 C. Detailed studies of one of the mutants (TS-223), which displayed the most extreme response to temperature, indicated that it also had reduced cloning efficiency at high temperature in

monolayer culture and a lower saturation density. Scanning electron microscopy revealed that at 40 C, confluent cultures of TS 223 consisted of a monolayer of generally flat polygonal cells; at 36 C, the cultures contained many patches of piled-up cells that were spherical and had rougher surface membranes. All of these cellular changes were reversible with higher or lower temperatures. TS lesions appear to reside in a host cell gene that modulates expression of the transformed phenotype. When the 36 C and 40 C cultures were at their plateaus in saturation density, there was a very low level of thymidine incorporation. A change to fresh medium at both 36 C and 40 C led to a burst of thymidine incorporation. While the TS mutants display minimal features of transformation complicating characterization, they may be useful in outlining minimal changes required for the expression of the transformed phenotype in epithelial cells.

171. DIFFERENTIAL EXPRESSION OF TRANSFORMATION IN RAT AND CHICKEN CELLS INFECTED WITH AN AVIAN SARCOMA VIRUS TS MUTANT.

Graf T, Friis RR
Duke U. Med. Ctr., Durham, NC
Virology; 56(1):369-374 1973

Cells of the rat NRK line were infected with ts 339, a mutant of avian sarcoma virus B77, and clones were isolated which displayed the characteristics of transformed cells at 33 C. These cells reverted to a normal phenotype at 37 C. In comparison, chicken cells infected with the same mutant remained transformed at 37 C and acquired properties of normal cells only after temperature shift to 41 C. Several possibilities may serve as an explanation for the phenomenon observed: 1) the activity of the heat sensitive, virus specific molecule could be affected by factors in the microenvironment such as pH or the concentrations of certain ions, which could differ in avian and mammalian cells; 2) there could be a difference in receptor sites for the heat sensitive molecule required for transformation; and 3) since avian sarcoma viruses merely establish an abortive infection in mammalian cells, it appears possible that they induce less transforming molecules in mammalian than in chicken cell. The 'leakiness' of the temperature sensitive molecule might thus be greater in the chicken system and could be reflected in an increased heat resistance of the transformation process in these cells.

172. A NOVEL TYPE OF CELLULAR VARIANT WITH ALTERED EXPRESSION OF VIRUS-INDUCED CELL TRANSFORMATION.

Graf T, Beug H
Max-Planck-Institut für Virusforschung, Tübingen, W. Germany
Virology; 72(1):283-286 1976

Transformed variants were obtained from rat NRK cells infected by temperature-sensitive avian sarcoma virus mutants and grown at nonpermissive temperatures. The finding of transformed variants in this population could be explained either by a back mutation of the virus or by an alteration of the host cell. Virus recovered from all the variants was still temperature sensitive for transformation as determined by chicken cells. This finding indicates the occurrence of a host cell modification. (11 refs)

173. CHARACTERIZATION OF SIMIAN CELLS TRANSFORMED BY TEMPERATURE-SENSITIVE MUTANTS OF SIMIAN VIRUS 40.

Noonan CA, Brugge JS, Butel JS
Department of Virology and Epidemiology, Baylor College of Medicine, Houston, TX 77030
J Virol; 18(3):1106-1119 1976

Seven lines derived from primary African green monkey kidney cells, which had survived lytic infection by wild-type simian virus 40 (SV40) or temperature-sensitive mutants belonging to the A and B complementation groups, were established. These cultures synthesize SV40 tumor (T) antigen constitutively and have been passaged more than 60 times in vitro. The cells released small amounts of virus even at high passage levels but eventually became negative for the spontaneous release of virus. Virus rescued from such 'nonproducer' cells by the transfection technique exhibited the growth properties of the original inoculum virus. Four of the cell lines were tested for the presence of altered growth patterns commonly associated with SV40-induced transformation. Although each of the cell lines was greater than 99% positive for T antigen, none of the cultures could be distinguished from primary or stable lines of normal simian cells on the basis of morphology, saturation density in high or low serum concentrations, colony formation on plastic or in soft agar, hexose transport, or concanavalin A agglutinability. However, the cells could be distinguished from the parental green monkey kidney cells by a prolonged life span, the presence of T antigen, a resistance to the replication of superinfecting SV40 virus or SV40 viral DNA, and, with three of the four lines, an ability to complement the growth of human adenovirus type 7. These properties were expressed independent of the temperature of incubation. These results indicate that the presence of an immunologically reactive SV40 T antigen is not sufficient to ensure induction of phenotypic transformation and suggest that a specific interaction between viral and cellular genes and/or gene products may be a necessary requirement. (Author Abstract)

174. ISOLATION OF HEAT- AND COLD-SENSITIVE MUTANTS OF CHINESE HAMSTER LUNG CELLS AFFECTED IN THEIR ABILITY TO EXPRESS THE TRANSFORMED STATE.

Miyashita K, Kakunaga T
Res. Inst. for Microbial Diseases, Osaka Univ., Osaka 565
Japan
Cell (Camb); 5(2):131-136 1975

A clone of spontaneously transformed Chinese hamster lung cells (actively growing) was exposed to 3'-methyl-N'-nitro-N-nitroso-guanidine (MNNG) (0.2 microg/ml for eight hr at 34.5 C), and six heat-sensitive and three cold-sensitive mutants were isolated after selection for inability to form colonies in soft agar at 39.5 C and 34.5 C, respectively. The heat-sensitive mutants had growth characteristics of transformed cells at 34.5 C, but exhibited a normal phenotype at 39.5 C. By contrast, cold-sensitive mutants displayed the characteristics of the normal cells at 34.5 C and converted to a transformed phenotype at 39.5 C. Temperature shift experiments showed that the colony-forming ability of both types of mutants was fully reversible. All of the mutants were able to grow well at both permissive and nonpermissive temperatures when grown on the surface of plastic dishes. The mutants provide many advantages for comparative studies of the transformed phenotype, including a high plating efficiency, relative genetic homogeneity, and stable temperature-dependent properties.

175. SOMATIC MUTATION AS THE BASIS FOR MALIGNANT TRANSFORMATION OF BHK CELLS BY CHEMICAL CARCINOGENS.

Bouck N, Di Meyorca G
Dept. Microbiology, Univ. Illinois Medical Center,
Chicago, IL 60612
Nature (Lond); 264(5588):722-727 1976

The somatic mutation as the basis for the malignant transformation of BHK cells by chemical carcinogens is studied. The established quasi-diploid hamster line BHK21/cl 13 and the carcinogens nitrosomethylurea (NMU) and 4-nitroquinoline-1-oxide (NQO) were used. NMU transformed efficiently. The max transformation frequency observed was 3.8×10^{-5} transformant per treated cell, 4×10^{-4} transformant per survivor, an almost 300-fold increase over the spontaneous frequency. NQO transformed effectively, even at doses where survival was unaffected. The max transformation frequency observed was 4×10^{-6} per treated cell and 2.2×10^{-5} per survivor, a 30-fold increase over the spontaneous frequency. Transformed colonies resulting from the carcinogen treatments were selected, purified by recloning, and split to high (38.5 C) and low (32 C) temperatures where they were grown and tested for the expression of the normal phenotype assessed as a significant depression in absolute and relative plating efficiency in soft agar. Some clones remained fully transformed at both temperatures. Fifty-three percent of the NMU-transformed clones and 55% of the NQO-transformed clones displayed this unconditional phenotype. The remainder of clones tested expressed the normal phenotype at one of the temperatures and the transformed phenotype at the other. The majority of these temperature restricted clones were transformed at the high temperature, normal at the low, as was expected, since transformants were initially seeded as clones growing in agar at 38.5 C. The frequency with which the cell line Me2N4 reverted to normal following ethylmethane sulfonate (EMS) mutagenesis was determined by growing EMS-treated cells at 38.5 C in methylcellulose containing 5-fluorodeoxyuridine to enrich for normal revertants. The number of revertants induced by EMS in the original population was estimated to be 4×10^{-6} per treated cell, 1×10^{-5} per survivor. The chemically-induced BHK transformants were similar to many well documented somatic cell mutants. The most reasonable explanation for these transformants is that they result from a somatic mutation. (56 Refs)

C. Effect of the Host Mutations/Variations on the Expression of Viral Functions

176. TRANSCRIPTION OF SIMIAN VIRUS 40. II. HYBRIDIZATION OF RNA EXTRACTED FROM DIFFERENT LINES OF TRANSFORMED CELLS TO THE SEPARATE STRANDS OF SIMIAN VIRUS 40 DNA.

Ozanne B, Sharp PA, Sambrook J
Cold Spring Harbor Lab., N.Y.
J Virol; 12(1):90-98 1973

The amount of simian virus 40 (SV40) DNA present in various SV40-transformed mouse cell line and 'revertants' isolated from them was determined. The number of viral DNA copies in the different cell lines ranged from 1.35-8.75 copies/diploid quantity of host DNA and from 2.2-14 copies/cell. The revertants had the same number of viral DNA copies/diploid quantity of mouse cell DNA as their parental cell lines; however, they showed an increased number of viral DNA copies/cell due to their increased amount of DNA. By using separated strands of SV40 DNA, the extent of each DNA strand transcribed into stable RNA species was determined for the transformed and 'revertant' cell lines. From 30-80% of the 'early' strand and from 0-20% of the 'late' strand

was present as stable RNA species in the cell lines tested. There was no alteration in the pattern of the stable viral RNA species present in three concanavalin A-selected revertants, whereas in a fluorodeoxyuridine-selected revertant there was less viral-specific RNA present in the cells.

177. SIMIAN VIRUS 40-PERMISSIVE CELL INTERACTIONS: SELECTION AND CHARACTERIZATION OF SPONTANEOUSLY ARISING MONKEY CELLS THAT ARE RESISTANT TO SIMIAN VIRUS 40 INFECTION.

Wilson JH, DePamphilis M, Berg P
Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030
J Virol; 20(2):391-399 1976

A fraction of permissive cells survives simian virus 40 (SV40) infection. The frequency of such surviving cells depends only upon the concentration of infecting virus, both parental and progeny, to which the cells are exposed during the course of selection. Surviving clones, which can be freed of virus by cloning in the presence of SV40 antiserum, are indistinguishable from parental cells in their growth characteristics and display no SV40 T antigen; thus they are not transformed. Most surviving clones are less than 10% as susceptible as parental cells to SV40 infection; 5 to 10% are less than 1% as susceptible. None of these SV40-resistant clones is absolutely resistant to SV40 infection. Analysis of 16 independently arising resistant clones indicates that they all block SV40 infection at an early stage after adsorption and eclipse but before full uncoating. Viral mutants have been isolated that partially overcome the block to infection in these cells; these host range viruses plaque on resistant lines fivefold more efficiently than wild-type SV40 and have a characteristic plaque morphology. Fluctuation analysis indicates that resistant cells arise spontaneously during the growth of normally susceptible permissive cells. Thus, SV40-resistant cells are selected for, not induced by, SV40 infection. (Author Abstract)

178. REVERTANTS OF MOUSE CELLS TRANSFORMED BY MURINE SARCOMA VIRUS. III. METASTABLE EXPRESSION OF VIRUS FUNCTIONS IN REVERTANTS RETRANSFORMED BY MURINE SARCOMA VIRUS.

Fischinger PJ, Nomura S, Tuttle-Fuller N, Dunn KJ
Natl. Cancer Inst., Bethesda, MD
Virology; 59(1):217-229 1974

Mouse 3T3FL cells transformed by the Moloney murine sarcoma virus (MSV) (sarcoma positive, leukemia negative, S+L- cells) segregate spontaneous flat revertants. These S-L revertants do not release MSV upon superinfection with murine leukemia virus (MuLV) but are susceptible to a second cycle of single-hit MSV transformation. The behavior and nature of second-cycle MSV transformed revertants were followed by successive clonal generations. The second transformation by MSV resulted in a number of metastable states. Of these the general phenomenon of changeability from S+ S- of L- L+ was striking. Observations on the metastable types are compatible with the interpretation that the second cycle of MSV transformation destabilizes cellular controls over the transforming genome.

179. THE PERSISTENCE AND EXPRESSION OF VIRUS-SPECIFIC DNA IN REVERTANTS OF ROUS SARCOMA VIRUS-TRANSFORMED BHK-21 CELLS.

Deng CT, Boettiger D, MacPherson I, Varmus HE
Dept. Microbiol., Univ. California, San Francisco, CA
Virology; 62(2):512-521 1974

Clones of Rous sarcoma virus-transformed baby hamster kidney cells and subclones reverted to normal phenotype were

found to contain one copy of integrated virus-specific DNA/diploid cell. In the transformed clones, virus-specific DNA was transcribed into RNA at low levels similar to those observed in other nonpermissive RSV-transformed cells. Virus-specific RNA concentrations were still lower in the revertant clones. The amount of virus-specific RNA in these clones correlated with the frequency with which Rous sarcoma virus could be rescued after fusion with chick embryo cells. Treatment of a revertant clone with 5-bromodeoxyuridine (25 microg/ml) increased the concentration of viral RNA and the frequency of rescue. These results suggest that control of viral gene expression in reverted cells is at least partially regulated at the transcriptional level.

180. ACTIVATION AND REPRESSION OF VIRUS EXPRESSION IN MAMMALIAN CELLS INFECTED BY ROUS SARCOMA VIRUS. (PP. 1169-1172)

Boettiger D
Tumor Viruses Cold Spring Harbor Symposia on Quantitative Biology; Cold Spring Harbor Lab. (Cold Spring Harbor, NY): vol. 39, 1975

Studies with transformed and nontransformed cells infected by Rous sarcoma virus were examined for frequency of virus rescue. It was found that nontransformed cells and revertants produced very few foci but that transformed cells produced a higher number. If infected cells contained 1 genome equivalent per cell, about 1 focus per 50 heterokaryon viral genomes was obtained. Revertants contained less than one-third genome equivalent per cell; they appear to have reverted because of a repression of viral RNA synthesis by the cell or by an alteration in viral RNA processing. Thus the control of viral expression appears to be at the level of RNA synthesis.

181. CHARACTERISTICS OF VIRUS-SPECIFIC RNA IN AVIAN SARCOMA VIRUS-TRANSFORMED BHK-21 CELLS AND REVERTANTS.

Deng CT, Stehelin D, Bishop JM, Varmus HE
Dept. Microbiology, State Univ. New York at Stony Brook, Stony Brook, NY 11794
Virology; 76(1):313-330 1977

Several characteristics of avian sarcoma virus (ASV) RNA transcripts were compared in transformed and reverted clones of baby hamster kidney (BHK) cells using hybridization with highly labeled, single-stranded, complement DNA (cDNA) synthesized by the DNA polymerase of ASV (ASV cDNA) to detect virus-specific RNA. Viral transcripts in both transformed and reverted clones contained poly(A) sequences and were associated with polyribosomes. In both clones viral RNA was three times more abundant in the cytoplasm than in the nuclei. In the transformed clone, whole-cell RNA contained 35S and 24S ASV RNA, but only 24S ASV RNA was detected in the cytoplasm; in the reverted clones, 24S ASV RNA was present in whole-cell RNA and in poly(A)-containing cytoplasmic RNA, but the amounts of viral RNA were too low to determine whether they also contained 35S ASV RNA. By using a hybridization reagent specific for nucleotide sequences required for transformation by ASV (cDNAsarc), it was shown that polyribosomal RNA in both transformed and reverted cells contains transformation-specific sequences. The concentration of these sequences, however, reflects the overall concentration of viral RNA in the cells. Therefore, no qualitative difference could be found in the pattern of viral gene expression in these phenotypically varied cells. A reduction in the concentration of viral RNA may be the only change in viral gene expression in the reverted cells. (55 Refs)

182. CELL LINE DERIVED FROM BALB/3T3 THAT IS TRANSFORMED BY MURINE LEUKEMIA VIRUS: A FOCUS ASSAY FOR LEUKAEMIA VIRUS.

Hackett AJ, Sylvester SS
Sch. Publ. Hlth., U. California, Berkeley, CA
Nature New Biol; 239(93):164-166 1972

A new cell line, designated UCI-B, has been derived from BALB/3T3 cells. The line consisted of polygonal cells which grow as flat, smooth monolayers and which are contact inhibited. Unlike the standard BALB/3T3 line, UCI-B cells could be morphologically transformed by murine leukemia virus (MuLV). The UCI-B cells yielded MuLV after infection but did not produce sarcoma virus. Transformed cells lost contact inhibition and foci of piled-up cells developed on the monolayer. Three different stock pools of MuLV and one of Rauscher leukemia virus produced morphologically identical transformed foci. Infected UCI-B cells yielded typical type-C virus as detected by 3H-uridine labeling, RNA-directed DNA polymerase assay, electron microscopy, and infectivity assays. In addition to typical C-type budding virus, bizarre forms were also observed in separate vacuoles within the same cell. Whether this represented defective MuLV replication or defective rescue of a latent sarcoma virus genome was unclear. The production of transformed foci on UCI-B monolayers was more rapid than, and as efficient as, XC-plaques on BALB/3T3.

183. INCREASED SARCOMA VIRUS RNA IN CELLS TRANSFORMED BY LEUKEMIA VIRUSES: MODEL FOR LEUKEMOGENESIS.

Peebles PT, Scholnick EM, Howk RS
Lab. Tumor Virus Genetics, Natl. Cancer Inst., Bethesda, MD 20014
Science; 192(4244):1143-1145 1976

The mechanism by which S+L- mink cells undergo transformation by the replicating mammalian-type C viruses was studied by means of a complementary DNA probe that represented the sarcoma specific sequences of the Moloney sarcoma virus. The morphologically flat revertant of mink cells nonproductively infected with Moloney sarcoma virus exhibited contact inhibition and lacked detectable sarcoma virus RNA. Superinfection by the usually nontransforming type C mammalian leukemia causing viruses induced transformation and increased sarcoma virus RNA. The results suggest a model for leukemogenesis in animals by increasing, during replication of usually nontransforming leukemia viruses, the levels of RNA from potentially oncogenic cell or integrated virus transforming virus. (15 refs)

184. ONCORNAVIRUS EXPRESSION IN MOUSE-HUMAN HYBRID CELLS SEGREGATING MOUSE CHROMOSOMES (MEETING ABSTRACT).

Minna J, Gazdar A, Iverson GM, Marshall T
Natl. Inst. Health, Bethesda, MD
Proc Am Assoc Cancer Res; 15:128 1974

The usual pattern of chromosome segregation in human-mouse hybrid cells is retention of mouse and loss of human chromosomes. Recently, however, the opposite result was found, in that human chromosomes were retained and mouse chromosomes lost in hybrid cells made by fusing the SV40 transformed human mutant, VA2, to brain cells freshly derived from C57 Bl/1 mouse embryos. Such human-mouse hybrid cells would appear to be useful for study of genes important in mouse oncornavirus expression, since loss of chromosomes containing regulatory genes might result in alteration in the expression of endogenous virus, provided that structural and regulatory genes are located on different chromosomes. In the current study, mouse-human hybrid cell lines segregating mouse chromosomes were examined for their

ability to elaborate type C viruses. The culture fluid from one hybrid cell line contained type C virus particles in abundance. No virus was detected in the culture fluid from the parental cells and several other hybrid lines. The hybrid-cell virus (HNV-1) was found to possess several properties characteristic of a murine leukemia virus. The results demonstrate that mouse-human hybrid cells segregating mouse chromosomes can spontaneously express latent type C viruses.

V. STATE AND EXPRESSION OF THE VIRAL GENOME IN VIRUS TRANSFORMED CELLS

A. Virus Integration

185. CHANGES OF REGULATION OF HOST DNA SYNTHESIS AND VIRAL DNA INTEGRATION IN SV40-INFECTED CELLS.

Hirai K, Campbell G, Defendi V
Wistar Inst. Anat. Biol., Philadelphia, PA
Cold Spring Harbor Conf Cell Proliferation; 1:151-165 1974

Previous studies on simian virus 40 (SV40)-infected Chinese hamster embryo cultures demonstrated an alteration in control of DNA synthesis that was expressed in the appearance of polyploidy. The biological significance of polyploid cells was examined by monitoring (1) the fate of the viral genome and its relationship to host DNA, and (2) the degree of polyploidy in relation to the appearance of the transformed state. In SV40-infected Chinese hamster cells, induction of cell DNA synthesis and viral DNA integration showed a good concurrence in terms of order of appearance, dependence on the functioning viral genome and on culture conditions. However, viral DNA was integrated even when induction of cell DNA synthesis was abolished by drugs or by use of a lethal virus mutant. Thus, integration of viral DNA does not depend on replication of cell DNA and precedes, at least functionally, the induction of cell DNA synthesis. Reinitiation of cell DNA synthesis was demonstrated by the appearance of tetraploid metaphases at the time of first mitosis after infection and by the finding of heavy-heavy (HH) DNA when normal cycling through mitosis was inhibited. The total amount and the percentage of HH DNA (newly synthesized cell DNA) synthesized during the first 48 hr postinfection was directly proportional to the amount of integrated viral DNA. Chromosome analyses were performed on transformed colonies selected by the agar method and on transformed colonies selected by the morphological phenotype in monolayers. In both groups of colonies, the distribution of tetraploid frequency indicated that transformed colonies do not originate exclusively from infected, preexisting tetraploid cells. At a time when 30% of cells were T antigen-positive and 20% of metaphases were polyploid, an infected population was fractionated in an albumen gradient that separated cells on the basis of volume. Both polyploid cells and cells capable of producing transformed colonies were concentrated in the first two pooled fractions, which contained only a minority of the total cell population. Therefore, cell populations containing more tetraploid cells originate more transformed colonies than those containing few tetraploid cells. These results suggest that reinitiation of DNA synthesis may result from viral DNA integration, and that polyploid cells are the source of the transformed population.

186. SIMIAN VIRUS 40 INTEGRATION SITES IN THE GENOME OF VIRUS-TRANSFORMED MOUSE CELLS.

Prasad I, Zouzas D, Basilico C
New York Univ. Sch. Medicine, New York, N.Y. 10016
J Virol; 16(4):897-904 1975

To gain information on the specificity of simian virus 40 (SV40) integration in the genome of transformed cells, mouse 3T3 cells were transformed by a temperature-sensitive (ts) SV40 mutant, using high multiplicity of infection (MOI). Transformed cells were superinfected with wild-type (wt) virus at high MOI. Clones were isolated and fused with permissive BSC-1 cells to promote virus rescue. All rescued viruses were of the ts type only. When the high-MOI transformants were infected with 3H-labeled wt SV40, the amount of radioactivity associated with their nuclear fraction was found to be similar to that of 3T3 cells. 3T3 cells were then transformed by ts SV40 at low MOI and superinfected by wt virus at high MOI. Upon fusion with BSC-1 cells, most clones produced both ts and wt virus. These results suggest that the number of stable SV40 integration sites in the 3T3 genome is limited, since they can be saturated by transformation at high MOI. When the MOI is low, the sites are not saturated and a subsequent infection can lead to integration.

187. ISOLATION OF DOUBLE LYSOGENS FROM 3T3 CELLS TRANSFORMED BY PLAQUE MORPHOLOGY MUTANTS OF SV40 (MEETING ABSTRACT).

Dubbs DR, Kit S
Baylor Coll. Med., Houston, TX
Proc Natl Acad Sci USA; 65(3):536-543 1970

Simian virus 40 (SV40) rescued from 3T3 lines transformed independently by fuzzy (f) plaque strain SV40(mKS-U4) and small-clear (sc) plaque strain, SV40(mKS-U88), resembled the virus employed to initiate transformation. Both plaque types could be rescued when a mixture of the 2 viruses was used for transformation. Two clonal lines, after fusion with CV-1 cells, gave both plaque types of infectious centers; a third clonal line, 3T3(4-88)G-1, yielded f and sc plaque types of infectious centers and large-clear (lc) infectious centers resembling wild-type (SV40 clone 307L). Two secondary and 14 tertiary clones of 3T3(4-88)G-1 were isolated; these all gave f plaque type and clear infectious centers after fusion with CV-1 cells. When these infectious centers were picked and replated on CV-1 cells, the f and sc plaque types of SV40, were isolated.

188. CELL TRANSFORMATION: A STUDY OF REGULATION WITH TYPES 5 AND 12 ADENOVIRUS TEMPERATURE-SENSITIVE MUTANTS. (PP. 419-426)

Ginsberg HS, Ensinger MJ, Kauffman RS, Mayer AJ, Lundholm U

Tumor Viruses Cold Spring Harbor Symposia on Quantitative Biology; Cold Spring Harbor Lab. (Cold Spring Harbor, NY): vol. 39, 1975

The types 5 and 12 temperature sensitive (ts) mutants of adenovirus (Ad) that cannot replicate their DNA under nonpermissive conditions (39.5 C) are described. In addition, one that can make the major viral gene products but under nonpermissive conditions cannot transport its hexon protein into the nucleus for virion assembly is characterized. The following insights were achieved: (a) viral DNA replication requires at least two specific viral proteins; (b) hexon protein transport requires a nonhexon viral protein; (c) virions assembly cannot ensue if one of the viral gene products is defective; and (d) transformation is the consequence of abortive infections with many adenoviruses, but its incidence is increased after infection with one class of adenovirus ts mutants. Two models are presented to explain the last observation. In one model, the DNA-binding protein normal-

ly maintains the viral DNA in a specific conformation or attached to some cellular structure so that viral genome integration is prohibited and viral DNA replication is initiated and continues. In the absence of this protein integration is more commonly permitted. In the second model, viral genome integration into a host chromosome is a normal event in the initial stages of infection, even during a productive infection. The latter hypothesis suggests that in a normal productive infection the entire viral genome is excised and replicated: defective excision occurs in abortively infected cells, leaving genome fragments to initiate and maintain transformation.

189. PRESENCE OF A HERPES SIMPLEX VIRUS TYPE 1 GENOME FRAGMENT IN HSV-TRANSFORMED CELLS. (PP. 415-419)

Kraiselburd E, Weissbach A

Oncogenesis and Herpesviruses II Part I: Biochemistry of Viral Replication and In Vitro Transformation
Proceedings of a Symposium held in Nuremberg, Federal Republic of Germany, 14-16 October 1974; International Agency for Research on Cancer (Lyon, France): IARC Scientific Publications, No. 11, 1975

LTK- cells infected with UV-irradiated herpes simplex virus (HSV) produce transformants that contain a thymidine kinase (TK) activity not found in the parental LTK- line. One of these (TK1) transformants (clone 139) was analyzed for the presence of the HSV genome. Reassociation kinetics studies with iodinated HSV DNA of specific activity of about 9×10^7 cpm/ug have established that there are approximately six copies of a fragment comprising about 15% of the HSV genome in HSV-transformed clone 139. Neither the parental LTK- nor a "revertant" cell line (clone 139 BUDR) obtained from clone 139 showed any detectable HSV-specific sequences. Analysis of data on RNA²²-125 I-HSV DNA reassociation kinetics indicated that perhaps 5% of the HSV genome is transcribed in HSV-transformed clone 139. Possibly, transcription of 10 of the 15 genes is necessary for the maintenance of the transformed state. These results indicate that transformation is probably maintained by the presence of only a fraction of the HSV genome in the TK1 clones.

190. NONPRODUCER CLONES OF MURINE SARCOMA VIRUS TRANSFORMED BALB/3T3 CELLS.

Aaronson SA, Rowe WP

Natl. Cancer Inst., Natl. Inst. Hlth., Bethesda, MD
Virology; 42(1):9-19 1970

Cultures of mouse embryo cells (BALB/c) were inoculated with murine sarcoma virus (2×10^5 virus-containing cells/culture dish), and 5 days later cultures were assayed for murine sarcoma virus foci; of 20 foci selected at limiting murine sarcoma virus solution, 18 released both murine sarcoma virus and murine leukemia virus. Two focus-derived lines, however, showed no physical or biological evidence of virus production and contained no evidence of antigens of the murine sarcoma-leukemia complex. These lines had altered properties in tissue culture and in vivo and were morphologically indistinguishable from virus releasing murine sarcoma virus-transformed mouse lines. The addition of helper murine leukemia virus resulted in the rescue of the murine sarcoma virus genome with host range and neutralization characteristics of the murine leukemia virus used to rescue it. These findings indicate that virus production and release is not necessary for the maintenance of the transformed state in mouse cells and may suggest that murine sarcoma virus is capable of initiating transformation without murine leukemia virus. The sarcoma genome could be passed from cell to daughter cell for more than 100 generations in the nonproducer lines, in the absence of any observable virus expression.

191. EFFICIENT RELEASE OF MURINE XENOTROPIC ONCORNAVIRUS AFTER MURINE LEUKEMIA VIRUS INFECTION OF MOUSE CELLS.

Fischinger PJ, Nomura S
Natl. Cancer Inst., Bethesda, MD
Virology; 65(1):304-307 1975

The theory that murine oncornavirus stocks could become contaminated with a virus group of a different host range and neutralization properties (even if previously passaged murine sarcoma virus or murine leukemia virus was composed of only one virus group) was examined. The virus progeny from a single cycle of Moloney murine leukemia virus infection of either normal or several types of murine sarcoma virus-transformed outbred or inbred mouse cells was investigated. The infecting Moloney murine leukemia virus derived from chronically infected 3T3FL cells was free of detectable xenotropic virus, but its ecotropic content was more than 106 focus-inducing U/ml in mouse S+L- cells. No cell line released free murine xenotropic oncornavirus spontaneously. Chemical induction with iododeoxyuridine released low amounts of murine xenotropic oncornavirus from normal BALB/c 3T3 cells and significantly more from K-murine sarcoma virus-transformed nonproducer cells. None of the outbred lines released any detectable murine xenotropic oncornavirus after chemical induction. Infection with Moloney murine leukemia virus resulted in the release of murine xenotropic oncornavirus from normal 3T3FL cells and wild mouse SC-1 cells but not from the revertant MISR derived from S+L- 3T3FL cells. BALB/c 3T3 cells yielded low titers of murine xenotropic virus on occasion after ecotropic virus infection. After murine leukemia virus infection, K-BALB cells produced a higher titer of murine xenotropic virus than seen by any other method of induction (more than tenfold greater than after iododeoxyuridine induction). A high titer of murine xenotropic virus (103 focus-inducing U/ml) was produced by S+L- mouse cells after murine leukemia virus infection; these same cells were not at all inducible for murine xenotropic virus by iododeoxyuridine. This virus was also completely neutralized by antisera from normal old NZB mice that were specific for murine xenotropic virus and that did not neutralize standard ecotropic types of murine leukemia virus. It is concluded that the murine xenotropic virus must have come from within the mouse cell because repeated attempts at infection of 3T3, S+L-, or revertant mouse cells with more than 105 focus-inducing Units of murine xenotropic virus resulted in an immediate loss of infection and no reappearance despite several blind passages.

B. Regulation of Induction of Loss of Viral Genomes

192. REVERSION IN POLYOMA-TRANSFORMED CELLS: RETRANSFORMATION, INDUCED ANTIGENS AND TUMORIGENICITY.

Marin G, MacPherson I
Int. Lab. Gen. Biophys, Naples, Italy
J Virol; 3(2):146-149 1969

The reversion (with potential for retransformation) of polyoma virus (PV)-induced transformation, seen in chromosomal segregants from a 'hybrid' clone of BHK21/13 hamster fibroblasts, was attributed to a loss of PV genes from the transformed cells. These cells lost their tumorigenicity; PV-specific complement-fixing antigen was absent, and the induced transplantation antigen was reduced or absent.

193. SEGREGATION OF MORPHOLOGICAL REVERTANTS IN POLYOMA-TRANSFORMED HYBRID CLONES OF HAMSTER FIBROBLASTS.

Marin G
Imp. Cancer Res. Fund, London, England
J Cell Sci; 9:61-69 1971

A series of hybrid clones was obtained by crossing untransformed B1 baby hamster kidney fibroblasts (BHK 21) with polyoma virus-transformed T6 clones, and by crossing untransformed T6 clones with transformed B1 clones. The B1 clone was resistant to 10^{*-4} M 5-bromodeoxyuridine and the T6 clone was resistant to $3 \times 10^{*-6}$ M 6-thioguanine (TG). The frequency of parental type segregants (i.e., cells resistant to TG or 5-bromodeoxyuridine) was measured in all hybrid clones and resistant colonies were scored for the presence of morphological revertants. Chromosomal segregants were produced by selecting for resistance to TG. Morphological revertants appeared among TG-resistant segregants in a minority of hybrid clones. In this system, morphological reversion arose independently of TG-resistance. Transformed and revertant colonies were isolated and grown up individually; each clone was tested for its ability to grow in agar medium and for the presence of polyoma virus-specific complement-fixing (CF) antigen. All revertant clones grew in agar with a very low efficiency. Polyoma virus-specific CF antigen was still present in some revertant clones; in other revertant clones, CF antigen was reduced to traces or was absent. The production of revertants appeared to be limited to an early phase in the life-history of the hybrid line. Morphological revertants showed a reduced chromosomal complement. Findings confirmed the hypothesis that morphological reversion occurred as a consequence of the loss of chromosomes concerned with the maintenance of the transformed phenotype.

194. REVERSION IN HYBRIDS BETWEEN SV40-TRANSFORMED HAMSTER AND MOUSE CELLS.

Wiblin CN, Macpherson I
Imperial Cancer Res. Fund Lab., London, England
Int J Cancer; 12(1):148-161 1973

The expression of the transformed phenotype was studied in segregants from a hybrid cell line resulting from the fusion of SV40-transformed BHK21 Syrian hamster cells (thioguanine resistant) and 3T3 mouse cells (bromodeoxyuridine resistant). Since the hamster cell parent was sensitive to bromodeoxyuridine, segregants lacking some chromosomes could be obtained by 'back-selection' in this drug. These segregants retained transformed characteristics, despite substantial loss of hamster chromosomes. Selected segregants were treated further with cytotoxic antibody produced against the hamster parent. The majority of the segregants isolated retained all transformed characteristics, even when the hamster chromosome complement was greatly reduced. Some derivatives showed properties intermediate between a transformed and an untransformed state. Two revertant clones were also isolated which lacked transformed characteristics and contained no metacentric (hamster) chromosomes. Unlike the other segregants, no SV40 virus could be recovered from these cells. The results obtained do not allow firm conclusions as to the location or number of integration sites for SV40 genomes in TG2 cells. Since all detectable hamster chromosomes must be eliminated before loss of T-antigen and reversion of transformed characteristics occurs, this might imply multiple sites of insertion, all functional. A polygenic determination of transformed phenotype could explain the finding that a/s 1-51 and its derivatives contain reduced amounts of T-antigen and have lower efficiency of plating in agar and/or on plastic. It might be concluded that several virus gene copies are present, and threshold amounts of gene pro-

duct are required for the full expression of all transformed characteristics. With a lowered gene product content, one or some characteristics are only partially expressed, or not at all.

195. REPLICATION OF THE RESIDENT NON-DEFECTIVE SV40 IN TRANSFORMED HUMAN CELLS SUPERINFECTED WITH DNA FROM A TEMPERATURE-SENSITIVE SV40 MUTANT.

Dubbs DR, Kit S
Baylor Coll. Med., Houston, TX
J Gen Virol; 19(1):103-111 1973

Cells of a strain of human embryonic lung cells transformed by SV40 and producing infectious SV40 (WI38 Val3A cells) spontaneously produced infectious virus which was temperature resistant (tr), i.e. replicated in monkey kidney cells at either 38.7 C or 33.5 C. Superinfection of WI38 Val3A cells with DNA from a temperature-sensitive (ts) SV40 mutant at the permissive temperature (33.5 C) did not enhance rescue of the resident tr SV40 genome. In related experiments, the competition between replicating superinfecting ts SV40 and endogenous tr SV40 was studied. It was determined that failure of superinfecting ts SV40 to enhance replication of resident tr SV40 was not due merely to a competitive advantage of ts SV40 over tr SV-40. 1-beta-D-arabinofuranosylcytosine treatment of WI38 Val3A cells did not enhance formation of the endogenous tr SV40. The experiments suggested that the resident SV40 is determined primarily by cellular rather than by superinfecting SV40 gene functions.

196. CIRCULAR DNA FROM HETEROKARYONS OF SV40-TRANSFORMED MOUSE AND AFRICAN GREEN MONKEY CELLS.

Yoshiike K, Watanabe S, Suzuki K, Uchida S
Natl. Inst. Health, 2-10-35 Kamiyosaki, Shinagawa-ku,
Tokyo 141, Japan
Jpn J Microbiol; 19(3):237-240 1975

In an attempt to explore a method for the study of Simian virus 40 (SV40) genome excision, closed circular DNA was extracted from heterokaryon cultures of SV40-transformed mouse and susceptible monkey cells and its length determined by electron microscopy. Dish cultures and coverslip cultures were prepared from incubation mixtures of African green monkey kidney (GMK) cells, transformed mouse 3T3-L or 3T3-M cells, and UV-inactivated Sendai virus. After 72 hr of cultivation, the coverslips were stained with fluorescent antibodies and circular DNA was extracted from the dish cultures. The circular DNA molecules from the heterokaryon cultures appeared to be heterogeneous and ranged in size from 1.0-3.0 microm. The molecules constituting the largest proportion of the population had a length similar to that of the viral genome. The viral DNA from transformed 3T3-M cells, which is believed to have been transformed by defective SV40, was approximately 12% shorter than that from 3T3-L cells containing the complete genome. V- and T-antigen positive cells were found in the heterokaryon cultures of GMK cells and transformed 3T3-L cells, but cultures of GMK and transformed 3T3-M cells contained only T-antigen positive cells. The method used in this study, if applied to various transformed cell lines including those transformed by defective deletion mutants, may provide information about the excision of the SV40 genome.

197. INDUCTION OF VIRUS MULTIPLICATION IN 3T3 CELLS TRANSFORMED BY A THERMOSENSITIVE MUTANT OF POLYOMA VIRUS. I. ISOLATION AND CHARACTERIZATION OF TS-A-3T3 CELLS.

Vogt M
Salk Inst. Biol. Studies, San Diego, CA
Mol Biol; 47(3):307-316 1970

Mouse embryo fibroblasts susceptible to polyoma virus infection (3T3 cells) were transformed by infection with a thermosensitive mutant of polyoma virus, Ts-a. This transformation, which affected relatively few cells, was attributed to the virus rather than to spontaneous transformation because no transformed cells (Ts-a-3T3) were observed in controls, Ts-a-3T3 cells were as susceptible as nontransformed 3T3 cells to killing by polyoma virus, and derivatives of Ts-a-3T3 cells that lost the ability to produce virus reverted to an almost normal phenotype. All Ts-a-3T3 lines isolated continued to produce small amounts of virus when grown at 38.5 C. The amount of virus increased with time and reached a max when cultures became confluent. This capacity to produce virus was shown to be a hereditary trait of Ts-a-3T3 cells rather than a result of continued reinfection. Virus production increased markedly when Ts-2-3T3 cultures were shifted to 31 C. By plating for infective centers, determining virus yields from single cells and the proportion of cells synthesizing viral capsid proteins, it was established that increased virus production was due to increased production by only a fraction of the cells. The proportion of virus-producing cells continued to increase as long as the culture was actively growing. It is suggested that conditions favorable for cell growth are essential to initiate viral replication.

198. INDUCTION OF VIRUS MULTIPLICATION IN 3T3 CELLS TRANSFORMED BY A THERMOSENSITIVE MUTANT OF POLYOMA VIRUS. II. FORMATION OF OLIGOMERIC POLYOMA DNA MOLECULES.

Cuzin F, Vogt M, Dieckmann M, Berg P
Pasteur Inst., Paris, France
J Mol Biol; 47(3):317-333 1970

The physical state and replication of viral DNA was analyzed when cultures of 3T3 cells, transformed with a thermosensitive mutant of polyoma virus (Ts-a), were shifted from 38.5 C, where very little virus is produced, 31 C, where virus production rises sharply after 36 hours, and back again to 38.5 C. Cultures continued to produce viral DNA for at least 24 hours after they were shifted back to 38.5 C. The kinetics of ³H-labeled thymidine incorporation into viral DNA can be explained if the Ts-a function is needed in a unique event which initiates viral DNA replication in the transformed cell. Sedimentation through cesium chloride gradients showed that viral DNA synthesized at 31 C consisted of monomer (M), and superhelical closed circular dimer (D) and trimer (T) DNA. The oligomers accounted for 40% of total viral DNA. All 3 of these DNA forms infected secondary mouse embryo cultures. Thus, both D- and T-DNA molecules contained at least 1 complete polyoma genome equivalent. DNA-DNA hybridization experiments confirmed the dimeric and trimeric nature of D- and T-DNA. However, the results of this test did not rule out the possibility that the oligomers contained unlabeled host DNA. Oligomers are not obligatory precursors of monomers and are not present in mature virions. Only small amounts of oligomers were synthesized after acute infection with either wild-type or Ts-a polyoma virus.

199. PROPOSED STRUCTURE OF TWO DEFECTIVE VIRAL DNA OLIGOMERS PRODUCED IN 3T3 CELLS TRANSFORMED BY THE TS-A MUTANT OF POLYOMA VIRUS.

Vogt M, Bachelier LT, Boice L
The Salk Institute, San Diego, CA 92112
J Virol; 17(3):1009-1026 1976

The various oligomeric viral DNA species produced at 32 C by two related sublines of ts-a-transformed mouse 3T3 cells were characterized. Results from the analysis of the cleavage products observed after digestion with restriction endonucleases from *Haemophilus parainfluenzae*; *Escherichia coli* RI, and *Haemophilus suis* are consistent with the assumption that in both sublines, the major oligomeric component is a dimer from which a segment of different length is deleted. The major oligomeric (27S) component in subline 1 was estimated to be 1.77 times the size of the viral monomer, and the major (25.5S) component in subline 15 was estimated to be 1.54 times the size of the viral monomer. These size estimates were confirmed by electron micrograph measurements. The larger oligomers produced by both sublines were found to be multiples of the major oligomeric component of each subline.

INDUCTION OF VIRUS SYNTHESIS IN POLYOMA-TRANSFORMED BHK-21 CELLS.

Folk WR
Imperial Cancer Res. Fund, London, England
J Virol; 11(13):424-431 1973

BHK-21 cells were transformed with polyoma virus mutants Ts-a and Ts-25 by using a temperature shift from 31 to 39 C at 5 days after infection so that rescuable transformants could be isolated. Clones which yielded virus after fusion with mouse cells were scored and maintained at 39 C in the presence of antipolyoma virus antiserum. Generally, no infectious viral deoxyribonucleic acid (DNA) could be found in Hirt supernatant fractions of these lines when maintained at 39 C, but DNA-DNA reannealing measurements detected two to six viral genomes per diploid cell genome in the nuclear DNA. Fusion with permissive cells was not necessary to induce the synthesis of infectious virus; cell lines shifted to 31 C produce the equivalent of 100 viral genomes per cell after 5 days. In some cell lines up to 1% of the cells formed infectious centers upon a shift to 31 C, and 100% of the subclones of a line were inducible. Growth at 31 C selected for a noninducible population which was still transformed.

201. POLYOMA GENOME IN HAMSTER BHK-21-C13 CELLS: INTEGRATION INTO CELLULAR DNA AND INDUCTION OF THE VIRAL REPLICON.

Folk WR, Bancuk JE
Department of Biological Chemistry, The University of Michigan Medical School, Ann Arbor, MI 48109
J Virol; 20(1):133-141 1976

When grown at 39.5 degrees C, BHK-21-C13 cells transformed by A gene mutants of polyoma virus contain viral sequences that are predominantly associated with cellular DNA pelleted in the Hirt lysis procedure. At this temperature, in cells that are inducible for viral DNA replication (Folk, 1973), the majority of the viral genomes are covalently joined with cellular DNAs containing repetitive sequences. Upon a shift to 31 degrees C, free viral genomes appear and are replicated. Coupled with the replication of the free viral genomes at 31 degrees C is an increase in the viral genomes associated with cellular DNA. (Author Abstract)

202. STATE OF THE VIRAL DNA IN RAT CELLS TRANSFORMED BY POLYOMA VIRUS. I. VIRUS RESCUE AND THE PRESENCE OF NONINTEGRATED VIRAL DNA MOLECULES.

Prasad I, Zouzas D, Basilico C
New York Univ. Sch. Medicine, New York, NY 10016
J Virol; 18(2):436-444 1976

When polyoma virus was interacted with a continuous line of rat cells, the infection did not cause virus multiplication but induced transformation. Transformed cells did not produce infectious virus, although in all clones tested virus could be rescued upon fusion with permissive mouse cells. Transformed rat cells contained, in addition to integrated viral genomes, 20 to 50 copies of nonintegrated viral DNA equivalents per cell (average). Nonintegrated viral DNA molecules were also found in cells transformed by the ts-a and ts-8 polyoma mutants and maintained at 33 C. These "free" viral DNA particles were not due to a virus carrier state, because the number of nonintegrated viral DNA molecules was unchanged when cells were grown in antipolyoma serum. Recloning of the transformed cell lines produced subclones, which also contained free viral DNA. Most of these molecules were supercoiled and were found in the nuclei of the transformed cells. The nonintegrated viral DNA is infectious; that its specific infectivity is about 100-fold lower than that of polyoma DNA extracted from productively infected cells suggests that these molecules contain a large proportion of defectives. This free viral DNA may have originated from an integrated template.

203. ISOLATION AND CHARACTERIZATION OF A HEAT-INDUCIBLE SIMIAN VIRUS 40 MUTANT.

Rothschild H, Lockwood MP
Department of Medicine, Louisiana State University, School of Medicine, New Orleans, LA 70112
J Virol; 19(2):374-381 1976

We have isolated a new type of temperature-sensitive mutant of simian virus 40 (SV40) that is capable of productive infection in permissive cells but not of maintenance of viral DNA integration in transformed cells at the conditional temperature. Virus development is induced when cells transformed by this mutant are shifted to temperatures above 39 degrees C, but it is not induced below this temperature. The plaque-purified, temperature-sensitive mutant virus confers heat inducibility to new host cells, indicating that the conditional function is a property of the viral genome. Unlike previously described temperature-sensitive SV40 mutants, in(ts)-1501 is capable of productive infection in permissive cells at the conditional temperature. The morphology, growth, and oncogenicity of in(ts)-1501-transformed cells at 37 degrees C are similar to those of cell lines transformed by wild-type SV40. HK10-c2(in(ts)-1501), a cloned cell line, transformed at 37 degrees C by the mutant virus, exhibits a transient increase in DNA synthesis before cell death at the conditional temperature. Many properties of in(ts)-1501 are analogous to those of the heat-inducible mutants of bacteriophages in which a heat-inactivated protein is responsible for the stable integration of the prophage in the bacterial chromosome. (Author Abstract)

204. CHARACTERIZATION OF A HEAT-INDUCIBLE SV40 MUTANT (MEETING ABSTRACT).

Rothschild H, Lockwood MP, Kasten G
LSU School of Medicine New Orleans, LA 70112
Proc Am Assoc Cancer Res; 17(1):48 1976

Temperature-sensitive (ts) mutants have been valuable tools for understanding the gene functions of oncogenic viruses. We have isolated a new type of temperature-sensitive mutant of SV40, SV40-ti1, which is activated from its integrated state

in transformed hamster kidney cells by incubation at 41 degrees C for 24 to 96 hours but is not activated spontaneously at temperatures below 38 degrees C. This indicates that a protein is necessary for maintenance of viral DNA integration. The plaque-purified, temperature-sensitive mutant virus confers heat inducibility to new host cells, indicating that the conditional function is a property of the viral genome. Unlike previously described temperature-sensitive SV40-mutants, SV40-ti1 is capable of lytic infection at the restrictive temperature. SV40-ti1-transformed cells are similar to cells transformed by wild-type SV40, being inducible at 37 degrees C by mitomycin C and UV irradiation and being tumorigenic in vivo. HK10-CL1(SV40-ti1) a cloned cell line, transformed by the plaque-purified mutant virus at 37 degrees C, exhibits transient increase in DNA synthesis prior to cell death at the restrictive temperature. Many properties of SV40-ti1 are analogous to those of the heat-inducible cI mutants of lambda phage, in which heat inactivates a repressor protein. (Author Abstract)

205. REVERSION OF MURINE SARCOMA VIRUS TRANSFORMED MOUSE CELLS: VARIANTS WITHOUT A RESCUABLE SARCOMA VIRUS.

Fischinger PJ, Nomura S, Peebles PT, Haapala DK, Bassin RH

Natl. Cancer Inst., Bethesda, MD
Science; 176(4038):1033-1035 1972

Extended cultivation of single colonies of murine sarcoma virus (MSV)-transformed mouse 3T3 cells (S+L-), which were negative for murine leukemia virus (MuLV) and which yielded sarcoma virus after super-infection with MuLV, resulted in the formation of spontaneous flat revertants from which MSV could no longer be rescued. The revertants were able to support MuLV growth and showed an enhanced sensitivity to MSV superinfection. Like normal cells, the flat revertants did not release RNA-dependent DNA polymerase activity into the supernatant fluid, nor did they have any detectable C-type particles. Because revertants could be obtained with high frequency from progeny of single transformed cells, each cell that contained the MSV genome seemed to have the capacity to suppress or eliminate an RNA tumor virus native to its species of origin.

206. ELIMINATION OF THE SARCOMA GENOME FROM MURINE SARCOMA VIRUS TRANSFORMED CAT CELLS.

Frankel AE, Haapala DK, Neubauer RL, Fischinger PJ
Natl. Cancer Inst., Bethesda, MD 20014
Science; 191(4233):1264-1266 1976

Evidence is presented for the loss of a transforming sarcoma genome in cloned, spontaneous revertant cell lines obtained from a Moloney murine sarcoma virus-transformed cat cell line. The revertant cell lines had no evidence of the sarcoma genome in the cell RNA or DNA as determined by RNA-complementary DNA or DNA-complementary DNA hybridizations and analysis by hydroxylapatite chromatography. This is the first report of loss of a transforming genome in a revertant cell line.

C. Expression of Viral Genes

207. REGULATION OF VIRAL TRANSCRIPTION AND TUMOR ANTIGEN EXPRESSION IN CELLS TRANSFORMED BY SIMIAN VIRUS 40.

Basilico C, Zouzas D

Department of Pathology, New York University School of Medicine, New York, NY 10016

Proc Natl Acad Sci USA; 73(6):1931-1935 1976

We have studied the expression of simian virus 40 (SV40) specific tumor antigen (T-antigen) and viral RNA in SV40-

transformed mouse 3T3 cells that are temperature-sensitive for the expression of the transformed phenotype (tsSV3T3). Although transformed by wild-type SV40, tsSV3T3 cells at 32 degrees behave like standard transformants, while at 39 degrees they became arrested in G1 after reaching saturation density or under conditions of serum starvation. Ts SV3T3 cells at 32 degrees or exponentially growing at 39 degrees are uniformly T-antigen positive. However, after G1 arrest at 39 degrees the majority of the cells becomes T-antigen negative. Induction of proliferation in the resting cultures results in the reappearance of T-antigen in most of the cells, concomitant with the induction of DNA synthesis. The reason for the disappearance of T-antigen from tsSV3T3 cells arrested in G1 seems to reside in a transcriptional control operating on the integrated viral DNA, since these cells contain no appreciable amounts of SV40 specific RNA. Viral RNA can be easily detected in cells growing at 32 degrees or at 39 degrees. The results suggest that transcription of the viral genome in SV40-transformed cells is cell-cycle-dependent. (Author Abstract)

208. SUPPRESSION OF THE TEMPERATURE-SENSITIVE CHARACTER OF ADENOVIRUS 12 EARLY MUTANTS IN MONKEY CELLS TRANSFORMED BY AN ADENOVIRUS 7-SIMIAN VIRUS 40 HYBRID.

Shiroki K, Shimojo H, Sekikawa K, Fujinaga K, Rabek J, Levine AJ

Inst. Medical Science, Univ. Tokyo, P.O. Takanawa, Tokyo, 108, Japan

Virology; 69(2):431-437 1976

Interactions of three temperature-sensitive, DNA replication-defective mutants of adenovirus 12 (Ad 12), tsA, tsB, and tsC, with Ad 7- and simian virus 40 (SV40)-coinfected cells, and in Ad 7-SV40 hybrid-transformed monkey (H5) cells, were investigated. Each class of virus mutant was temperature dependent for virus production, viral DNA synthesis and virion antigen in human embryonic kidney cells and in T22 cells (a monkey cell line transformed by defective particles of SV40). In H5 cells, however, the tsB and tsC mutants were not temperature sensitive for viral replication, while the tsA mutant retained its temperature sensitive phenotype. Wild-type Ad 7, but not wild-type SV40, was able to complement tsA, tsB and tsC for either infectious virus production or DNA replication. These observations suggest that Ad 7-specific functions present in the H5 cells complement or suppress the tsB and tsC gene functions. DNA-DNA reassociation kinetics and DNA-RNA hybridization competition experiments were performed, and demonstrated the presence and the transcription of at least a portion of the Ad 7 genome in the H5 cells.

209. TRANSFER OF THYMIDINE KINASE TO THYMIDINE KINASELESS L CELLS BY INFECTION WITH ULTRAVIOLET-IRRADIATED HERPES SIMPLEX VIRUS.

Munyon W, Kraiselburd E, Davis D, Mann J

Roswell Park Mem. Inst., Buffalo, NY

J Virol; 7(6):813-820 1971

Herpes simplex virus (HSV) suspensions were exposed to UV for 1-18 min in amounts of 23 ergs/mm2/second; irradiated virus was used to infect cultures of L cells which lacked thymidine kinase activity (Ltk- cells). The transformation of Ltk- to Ltk+ cells in virus-infected cultures was measured by the formation of colonies of Ltk+ cells in a supplemented growth medium. No colony formation was observed with uninfected L cells, or with cells infected with unirradiated HSV, but colony formation was seen with cultures infected with irradiated HSV; the maximum frequency of Ltk- to Ltk+ transformation was about 10-3. Cell lines of HSV-transformed Ltk+ cell lines contained 7-24 times as much thymidine kinase activity as did parental Ltk-

cells. Optimal formation of Ltk⁺-transformed cell colonies was seen in cultures infected with HSV which had been irradiated with UV for 9-12 min. The number of Ltk⁺-transformed colonies formed was proportional to the dose of virus. Substitution of bromodeoxyuridine for thymidine in the supplemented growth medium, which was infected with irradiated HSV, suppressed colony formation. A mutant strain of HSV which failed to induce thymidine kinase activity during lytic infection failed to cause the Ltk⁻ to Ltk⁺ transformation. This transformation may have been caused by the transfer of a viral gene to the Ltk⁻ cells; alternatively, the transformation may have been caused by the promotion of expression of a repressed cellular enzyme by a product of the viral gene.

210. EXPRESSION OF THE VIRAL THYMIDINE KINASE GENE IN HERPES SIMPLEX VIRUS-TRANSFORMED L CELLS.

Lin SS, Munyon W
Roswell Park Mem. Inst., Buffalo, NY
J Virol; 14(5):1199-1208 1974

The expression of thymidine kinase (TK) was compared in normal and herpes simplex virus (HSV)-transformed L cells. In asynchronously dividing cultures of L cells, Tk activity rose and declined rapidly and coordinately with DNA synthesis. When net cell increase stopped, TK activity was at a minimum. In contrast, TK activity of HSV-transformed cells remained at a minimum during rapid DNA synthesis and gradually increased as the rate of DNA synthesis decreased. When net cell increase stopped, TK activity was at a maximum. In synchronous cultures of L cells, TK activity rose and fell coordinately with the rate of DNA synthesis. In synchronous cultures of HSV-transformed cells, no increase in TK activity was observed during the period of rapid DNA synthesis, i.e., the S phase. These findings indicated that the viral TK genome in HSV-transformed cells was not placed under control of the cellular mechanisms which normally modulate the host cell TK gene. Lytic infection of HSV-transformed cells with a TK mutant of HSV-1 induced a 4- to 5-fold increase in viral TK. The TK of HSV-1 was induced in the HSV-1 transformed cells and HSV-2 in the HSV-2-transformed cells by this TK mutant. The same infection of normal L cells decreased the cellular activity by 80%. This stimulation, rather than inhibition, suggests that the viral gene in HSV-transformed cells retains some of its original viral characteristics.

211. HERPES SIMPLEX VIRUS GENE EXPRESSION IN TRANSFORMED CELLS I REGULATION OF THE VIRAL THYMIDINE KINASE GENE IN TRANSFORMED L CELLS BY PRODUCTS OF SUPERINFECTING VIRUS.

Leiden JM, Buttyan R, Spear PG
Department of Microbiology, University of Chicago,
Chicago, IL 60637
J Virol; 20(2):413-424 1976

In this paper we show that the expression of the herpes simplex virus type 1 (HSV-1) gene for thymidine kinase (tk) in HSV-transformed cells is subject to regulation by two viral products synthesized during productive infection of these cells with a tk⁺ mutant of HSV-1. The cell line used in this study is a derivative of tk-deficient mouse L cells that, after exposure to UV-inactivated HSV-1, had acquired the HSV-1 gene for tk (which we term a resident viral gene) and consequently expressed the tk⁺ phenotype (LVtk⁺ cells). Productive infection of these cells with HSV-1(tk⁺) at appropriate multiplicities caused significant enhancement of the viral tk activity. The results of several experiments allow us to conclude that this enhancement was due to increased synthesis of tk specified by the HSV-1 gene resident in the LVtk⁺ cells and that a specific protein made early after infection with HSV-

1(tk⁺) mediated the enhancement, probably by increasing the production of mRNA from the viral tk gene resident in the LVtk⁺ cells. Our data also indicate that another HSV-1(tk⁺) product acted to turn off tk synthesis. The finding that tk activity continued to increase for a longer time after infection of the LVtk⁺ cells at 2 PFU/cell than after infection at higher multiplicities suggested the synthesis of a product which inhibited tk synthesis and whose concentration reached critical levels earlier at higher multiplicities of infection. Inhibition of DNA synthesis after infection, a treatment that depresses the synthesis of late viral proteins, prolonged the synthesis of tk in LVtk⁺ cells infected at either 2 or 5 PFU/cell. Infection of the LVtk⁺ cells with HSV-2(tk⁺) resulted in only small increases in tk activity, indicating some type specificity in recognition of viral products that can modify the expression of the HSV-1 tk gene resident in these cells. (Author Abstract)

212. ENHANCED REPLICATION OF TEMPERATURE-SENSITIVE MUTANTS OF HERPES SIMPLEX VIRUS TYPE 2 (HSV-2) AT THE NONPERMISSIVE TEMPERATURE IN CELLS TRANSFORMED BY HSV-2.

Kimura S, Esparza J, Benyesh-Melnick M, Schaffer PA
Baylor Coll. Med., Houston, TX
Intervirology; 3(3):162-169 1974

To determine whether the herpes simplex virus type 2 (HSV-2) genetic information present within HSV-2-transformed cells could assist in the replication of temperature-sensitive (ts) mutants of HSV-2 at the nonpermissive temperature (38 C), the replication of eight ts mutants of HSV-2 was examined in normal, simian virus 40 (SV40)-transformed, and HSV-2-transformed hamster embryo fibroblasts (HEF). The wild-type (WT) virus replicated well in normal and HSV-2-transformed HEF; the mutant tsF3 did not replicate well in either cell type, but the growth of two other mutants (tsG4 and tsD6) was enhanced 10-fold and 6-fold, resp. in HSV-2-transformed as compared with normal HEF. Yields of tsG4 and tsD6 were maximal at 24 hr postinfection. Antiserum treatment of the infected cells immediately after adsorption had no effect on the yields of infectious virus or on the degree of enhancement of any of the mutants. The replication of two DNA⁺ and four DNA⁻ ts mutants was not markedly enhanced in HSV-2-transformed cells compared with normal HEF; the replication of the two DNA⁺ mutants, tsD6 and tsG4, was however, significantly enhanced in the HSV-2-transformed cells. No enhancement of yield was observed with any of the ts mutants in the SV40-transformed cells. The results suggest that resident, functional HSV genetic information in HSV-2-transformed cells assisted the replication of the ts mutants by complementation mechanisms.

213. COMPLEMENTATION OF TS MUTANTS BY A HERPES SIMPLEX VIRUS TS-TRANSFORMED CELL LINE.

Macnab JC, Timbury MC
MRD Virology Unit, Inst. Virology, Church St., Glasgow
G11 5JR, United Kingdom
Nature (Lond); 261(5557):233-235 1976

Complementation experiments were performed with ts mutants of herpes simplex virus (HSV)-2 in RE 1 cells. Studies with ts3, ts4 and ts10 showed evidence of complementation. Yields from transformed cells infected with ts4 regularly produced larger plaques than control cells. No significant increase in yield was seen when transformed cells were infected with ts1. Furthermore, the mutants of ts2, ts5, ts6, ts7, ts8 and ts9 presented no evidence of significant complementation. Further studies indicated that the complementation of HSV ts mutants by RE 1 cells was unlikely to be the result of culturing. Examination of the four mutants of ts1, ts3, ts4 and ts10 revealed that the mutations were all in different genes. (14 refs)

214. REGULATION OF HERPESVIRUS GENE EXPRESSION IN BIOCHEMICALLY TRANSFORMED CELLS: INVESTIGATIONS OF VIRUS-SPECIFIED REGULATORY FUNCTIONS.

Spear PG, Leiden JM

Dept. Microbiology, Univ. Chicago, 939 E. 57th St.,
Chicago, IL 60637

Bibl Haematol; (43):53-58 1976

Preliminary investigations of viral gene regulation were performed in a mouse L cell line that lacks a functional gene for thymidine kinase (tk) but carries the tk gene of herpes simplex virus type 1 (HSV-1). Synthesis of tk by LVtk+ cells was enhanced significantly by infection of LVtk+ cells with a mutant of HSV-1 lacking the tk gene (tk-HSV**1). This enhancement varied with the multiplicity of infection, and no enhancement was observed at the highest multiplicity (10 PFU/cell). Antiserum against HSV-1 infected rabbit serum neutralized at least 75% of the tk activity in extracts of Ltk-cells infected with tk+HSV-1, whereas the tk activity from uninfected wild-type L cells could be reduced by only 29% by the antiserum. The same antiserum neutralized 70% of the tk activity in extracts from uninfected LVtk+ cells and 80% of the tk from infected LVtk+ cells that were exhibiting maximum enhancement of activity, indicating that the enzyme detected in the cells before infection was antigenically similar to or identical with the HSV-1 tk. Infection with tk-HSV-2 did not result in enhanced tk activity. L cell tk was not enhanced by tk-HSV-1 infection. Puromycin (50 ug/ml, added at 7 hr after infection) abolished enhanced tk activity, suggesting that the enhanced activity was the result of a newly synthesized enzyme and not increased activity of the existing enzyme. This conclusion was supported by the reversible inhibition of enhanced activity with cyclohexamide, which inhibited activity; after its removal from the medium, enhanced activity comparable to the controls was observed.

VI. ANALYSIS OF TRANSFORMED CELL PROPERTIES USING VIRUS AND CELL MUTANTS

A. Surface Properties of Transformed Cells

215. AGGLUTINATION OF CELLS TRANSFORMED BY ROUS SARCOMA VIRUS BY WHEAT GERM AGGLUTININ AND CONCAVALIN A.

Burger MM, Martin GS

Dept. Biochem. Sci., Princeton U., N.J.

Nature New Biol; 237(70):9-12 1972

Cultured chick embryo fibroblasts transformed by the Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV) were tested for their ability to agglutinate in the presence of wheat germ agglutination (WGA) or concanavalin A (Con A). Transformed cells exhibited only a slight increase in agglutinability compared with untransformed controls. However, pretreatment of transformed cells with purified hyaluronidase (250 IU/ml, 15 min) led to a 10- to 20-fold enhancement of agglutination with WGA and to a 6-fold increase with Con A. Pretreatment of untransformed control cells with hyaluronidase had no effect. The patterns of inhibition of agglutination by various low molecular wt carbohydrates and by ovomucoid suggested that the binding sites affected by SR-RSV were qualitatively similar to those affected by transformation with SV40 or polyoma virus. Chick cells were infected with a temperature sensitive mutant of RSV (T5) and agglutinability was tested after hyaluronidase treatment. The expression of the surface alteration occurred only at the permissive temperature (36 C). T5-infected cells grown at 36 C and shifted to the nonpermissive temperature (41 C) lost their

agglutinability by 12 to 18 hr. When cells were shifted back to 36 C, agglutinability was regained. These results suggest that agglutinability of the RSV-infected cells was associated with transformation, and not with virus release, which occurred at both temperatures.

216. AGGLUTINATION OF CHICK EMBRYO FIBROBLASTS TRANSFORMED BY THE SR-RSV STRAIN OF THE ROUS VIRUS AND BY A THERMOSENSITIVE MUTANT OF THIS VIRUS BY CONCAVALIN A.

Biquard JM, Vigier P

Faculte des Sciences, Essonne, France

C R Acad Sci D (Paris); 274(1):144-147 1972

Concanavalin A, a plant seed agglutinin (lectin), agglutinates animal cells transformed spontaneously, by an oncogenic virus, or by chemical carcinogens. Cellular surface areas contained sugars (alpha-methyl-D-glucopyranoside, in the case of Concanavalin A) forms bonds with agglutinins. Fibroblasts from normal chick embryos, fibroblasts from chick embryos infected with the SR-RSV strain of the Rous virus, fibroblasts from chick embryos infected with a thermosensitive mutant of the Rous virus (FU-19) incubated at 37 C, and the same fibroblasts incubated at 41 C, were agglutinated with Concanavalin A and results were evaluated. Agglutination was determined by the Burger and Goldberg method based on the percentage of agglutinated cells. Normal cells were only weakly agglutinated, and only by very high concentrations of Concanavalin A. Agglutination of cells transformed by the Rous virus and its FU-19 mutant at 37 C was more marked, proceeded faster and occurred at lower lectin concentrations. Agglutination of cells infected with FU-19 at 41 C required much higher lectin concentrations; however, cells incubated at 41 C are more agglutinable than control cells. Because transformation and agglutinability are reversible, it is suggested that these related characteristics stem from the same viral gene or group of viral genes.

217. SURFACE CHANGES OF HUMAN CELLS PRODUCTIVELY INFECTED WITH HUMAN ADENOVIRUSES.

Salzberg S, Raskas HJ

Saint Louis U. Sch. Med., MO

Virology; 48(3):631-637 1972

Surface membrane changes were detected in human embryonic kidney (HEK) cells productively infected with various human adenoviruses as an increased agglutination in the presence of concanavalin A (Con A). A significant increase in the agglutination of HEK cells by Con A was seen following infection with a group C adenovirus (type 2). The increase was detected as early as five hr after infection and reached a maximum after 40 hr. Viral DNA synthesis, or events dependent upon viral DNA synthesis, was necessary for this effect as treatment of cells with cytosine arabinoside from 8 to 12 hr postinfection (time of maximum viral DNA replication) considerably reduced the extent of agglutination. Infection of HEK cells with a group A adenovirus (type 12), an oncogenic strain, failed to increase agglutinability. However, two adenovirus 12 cytotoxic mutants, which have lost the ability to transform permissive cells, did increase the agglutination of infected HEK cells. This result indicated that the ability to produce surface change during productive infection was not necessarily related to the oncogenicity of the virus.

218. CELL SURFACE PROPERTIES AND THE EXPRESSION OF SV40-INDUCED TRANSFORMATION.

Smith HS, Hiller AJ, Kingsbury EW, Roberts-Dory C
Sch. Pub. Hlth., U. California, Berkeley CA
Nature New Biol; 245(142):67-69 1973

Different clones of SV40 transformed mouse embryo BALB/3T3 line of cells express transformation in different ways. To examine the correlation between type of transformation and surface properties said to be characteristic of transformed cells, the different clones were tested for agglutinability by concanavalin A (con A) and for membrane staining by ruthenium red as seen in the electron microscope. The different clones consisted of untransformed BALB/3T3, standard transformants (growing in multiple layers), flat transformants (growing in factor-free medium but not in multiple layers), and flat revertants (similar to flat transformants but arising from standard transformants). The standard SV40 transformed lines agglutinated with con A much more extensively than did non-transformed controls or flat transformants. However, a flat revertant, selected for growth to high density, was agglutinated by con A. With regard to staining with ruthenium red, standard transformants were positive, albeit to varying degrees, as also were those flat transformants and revertants which continued to synthesize DNA at confluence, while the one flat transformant which did not synthesize DNA at confluence stained weakly like the non-transformed control. It was pointed out that although most of the flat transformants and revertants are not contact inhibited and thus differ from control cells, they neither agglutinate with con A nor have transplantation immunity antigen. Thus the loss of contact inhibition per se is not sufficient to express other alterations associated with tumorigenicity. The increased staining of the flat transformants and revertants by ruthenium red suggests that an increase of acid mucopolysaccharide on the cell membrane is related to loss of contact inhibition.

219. CONCAVALIN A AGGLUTINABILITY OF A TEMPERATURE-SENSITIVE MUTANT OF ROUS SARCOMA VIRUS.

Miki T, Kuwata T
Department of Microbiology, School of Medicine, Chiba University, Chiba, Japan
J Gen Virol; 33(1):25-30 1976

Concanavalin A (Con A) concentration dependence of agglutinability of a mutant of Rous sarcoma virus which is temperature-sensitive for transformation was examined. Con A agglutinability of the virion was quantitatively expressed by measuring radioactivities of 60 to 70S RNA extracted from Con A-agglutinated material. The mutant grown at a permissive temperature (35 degrees C) agglutinated at a significantly lower concentration of Con A, compared with that at a non-permissive temperature (40 degrees C). (Author Abstract)

220. INTERACTIONS OF CONCAVALIN A WITH CHICK EMBRYO FIBROBLASTS TRANSFORMED BY ROUS SARCOMA VIRUS. STUDY WITH AN RSV MUTANT THERMOSENSITIVE FOR TRANSFORMATION.

Marciani DJ, Okazaki T
Laboratory of Biochemistry and Chemistry Branch,
National Cancer Institute, National Institutes of Health,
Bethesda, MD 20014
Biochim Biophys Acta; 455(3):849-864 1976

The interactions between concanavalin A and chick embryo fibroblasts, normal and infected with Rous sarcoma virus (RSV-BH) or its thermosensitive mutant RSV-BH-Ta, have been studied. Normal chick embryo cells and RSV-BH transformed cells showed at 4 and 25 degrees C a similar

number of concanavalin A receptors per cell. Analysis of the binding data by the Scatchard relation showed that apparent changes in binding as a function of temperature are due to the thermodynamic properties of the process and not to endocytosis. The lectin receptors on the cell surface of normal and RSV-BH infected cells showed homogeneity in their binding properties. Chick cells infected with RSV-BH-Ta showed a lectin binding behavior that was dependent on the temperature at which the cells were grown. At the permissive temperature for transformation (37 degrees C), the binding process was similar to that observed for normal and RSV-BH infected cells. At the nonpermissive temperature (41 degrees C), the cells showed at least two sets of concanavalin A receptors. The new set of receptors on the cell surface had a lower lectin affinity than those observed in the same cells at 37 degrees C. Chick cells infected with RSV-BH showed an enhanced agglutinability by concanavalin A, as compared with normal cells. Cells infected with RSV-BH-Ta showed a reversal of the correlation between increased concanavalin A agglutinability and the transformed state. At the permissive temperature for transformation, the cells were not agglutinable, whereas at the nonpermissive temperature they presented agglutinability indexes as high as those observed with RSV-BH infected cells. This enhanced agglutinability observed with cells maintained at the nonpermissive temperature for transformation may be related to the new set of low affinity receptors present at 41 degrees C. (Author Abstract)

221. ARE PROTEASES INVOLVED IN ALTERING SURFACE PROTEINS DURING VIRAL TRANSFORMATION?

Hynes RO, Wyke JA, Bye JM, Humphries KC, Pearlstein ES

Proteases and Biological Control. Edited by E. Reich, D.B. Rifkin, E. Shaw. Cold Spring Harbor Conferences on Cell Proliferation, Cold Spring Harbor Lab, Cold Spring Harbor, NY (2):931-944 1975

Three theories for the absence from the surfaces and purified membranes of virally transformed cells of a large, external, transformation-sensitive (LETS) glycoprotein were examined: (a) lack of synthesis, (b) continued synthesis but faulty processing, and (c) lack of retention in the membrane. The LETS protein is present in transformed cells in a latent form (an incompletely glycosylated precursor molecule or a molecule of different size and mobility on gels) that is expressed during protein synthesis inhibition or incubation at a restrictive temperature. After transformation or the addition of transformed cells to normal cells, an accelerated turnover of the LETS protein is observed, which suggests a role for proteases in altering cell surfaces after transformation. Activation of plasminogen is not required for the absence of LETS protein, but plasminogen activator or other transformation proteases may well be involved. Various cells were used; i.e., NIL8, normal hamster fibroblasts; NIL-HSV6, NIL8 cells transformed by hamster sarcoma virus; and temperature-sensitive Rous sarcoma virus mutants of chicken embryo fibroblasts from Brown Leghorn embryos. The LETS protein was identified by lactoperoxidase-catalyzed iodination and SDS-polyacrylamide gel electrophoresis.

222. PLASMA MEMBRANE PROTEINS OF NORMAL AND ROUS SARCOMA VIRUS-TRANSFORMED CHICK-EMBRYO FIBROBLASTS.

Wickus GG, Robbins PW
Dept. Biol., Massachusetts Inst. Technol., Cambridge, MA
Nature New Biol; 245(142):65-67 1973

To compare plasma membrane proteins of normal and transformed cells, cultures of chick embryo fibroblasts (CEF)

infected with the thermosensitive mutant (Ts-68) of the Schmidt-Ruppin strain of Rous sarcoma virus, subgroup A (SR-RSV-A), were incubated at a permissive temperature of 36°C to produce fully transformed cells or subsequently, at a non-permissive temperature of 41°C to reverse the morphology to that of normal cells. The two different cultures were labeled with ³⁵S L-methionine and plasma membrane vesicles were prepared from each. The vesicle preparations were then disrupted in sodium dodecyl sulfatemercaptoethanol and subjected to electrophoresis on polyacrylamide slab gels. Positions of the labeled polypeptides on the slabs were determined by autoradiography. Of 26 polypeptides seen on the gel for the cells with normal morphology, one was greatly reduced in the gel for the cells with transformed morphology. Additional studies were made with cell ghosts from the two different cultures and with membrane vesicles from uninfected cultures and cultures transformed by wild type RSV. The results all showed that the disappearance of only one polypeptide component was the major transformation specific alteration in plasma membrane preparations from RSV-transformed chick cells.

223. ROUS SARCOMA VIRUS TRANSFORMATION OF THE CHICK CELL SURFACE.

Wickus GG, Branton PE, Robbins PW
Dept. Biol., Massachusetts Inst. Technol., Cambridge, MA
Cold Spring Harbor Conf Cell Proliferation, 1:541-546
1974

To determine the role of transformation-induced chemical and physical cell surface changes in the loss of normal growth control by these cells, the transformation of chick fibroblasts by Rous sarcoma virus (RSV) was studied. Secondary chick embryo fibroblasts were infected with TS-68, a temperature-sensitive mutant of the Schmidt-Ruppin strain of RSV. These cells showed normal phenotypes at 41°C and the RSV-transformed phenotypes at 36°C. The infected cells were pulse-labeled with (³⁵S)methionine immediately before and for 3 hr periods after a shift in temperature from 41 to 36°C. Two major changes occurred in the labeling pattern in association with transformation, reduction in the synthesis of a 45,000-dalton protein found in normal chick cells during the period 3-6 hr after the induction of transformation and a rapid decrease in another protein component which was complete within the first 3 hr after the induction of transformation. The latter protein may be a cell surface protein bound loosely to the external face of the cell. Its electrophoretic properties were similar to those of the major protein of the chick cell surface, which can be iodinated by the lactoperoxidase-125I system. It is possible that this component is still made after transformation but that it is hydrolyzed rapidly by transformation-specific proteolytic enzymes; however, these data do not provide evidence for or against this hypothesis.

224. THE POSSIBLE CORRELATION OF GROWTH RATE AND EXPRESSION OF TRANSFORMATION WITH TEMPERATURE-DEPENDENT MODIFICATION IN HIGH-MOLECULAR-WEIGHT MEMBRANE GLYCOPROTEINS IN MAMMALIAN CELLS TRANSFORMED BY A WILD-TYPE AND BY A THERMOSENSITIVE MUTANT OF AVIAN SARCOMA VIRUS.

Reiber M, Irwin JC
Venezuelan Inst. Sci. Invest., Cent. Microbiol. Cell Biol.,
Caracas, Venezuela
Cancer Res; 34(2):3469-3473 1974

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to study the plasma membrane fractions of normal rat kidney cells transformed by a temperature-sensitive derivative of Rous sarcoma virus (NT2-Kr). The ts-NT3-KR cells showed the transformed morphology at 33°C and the untransformed phenotype at 37°C, as well as a temperature-

dependent growth in agar. Over 20 protein components were contained in the plasma membrane fractions of the cells grown at both 33 and 37°C. However, a selective increase in a protein with an apparent molecular wt of about 48,000 was evident in cells in which the transformed phenotype was expressed at 33°C. In addition, a 250,000-molecular wt glycoprotein component present in preparations from the cells grown at 37°C was diminished or absent in the plasma membranes isolated from the cells grown at 33°C. A similar study of normal rat kidney cells transformed by the wild-type avian virus revealed no temperature-mediated differences in the morphology or transformed properties of the cells grown at 33 and 37°C. However, there was a marked decrease in a 250,000-molecular wt protein in the cells grown at 37°C compared with those grown at 33°C. These cells showed more rapid growth and tended to reach a higher saturation density at 37°C compared with 33°C. Thus, the changes in glycoprotein content appear to depend on the growth rate of the transformed cells as well as on the expression of transformation.

225. ALTERATIONS IN MEMBRANE POLYPEPTIDES OF CHICK EMBRYO FIBROBLASTS INDUCED BY TRANSFORMATION WITH AVIAN SARCOMA VIRUSES.

Isaka T, Yoshida M, Owada M, Toyoshima K
Fac. Pharm. Sci., Univ. Tokyo, Japan
Virology; 65(1):226-237 1975

Membrane proteins of chick embryo fibroblasts (CEF) transformed with various strains of avian sarcoma viruses were analyzed by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels and compared with those of untransformed cells. Four changes were consistently detected in CEF transformed with B77, the Prague strain of Rous sarcoma virus (PR-RSV) or the Schmidt-Ruppin strain of RSV (SR-RSV). These included the appearance of a polypeptide band of about 90,000 molecular wt, an increase in amount of a 79,000 dalton polypeptide, and significant decreases in a protein and polypeptide of 200,000 and 50,000 daltons, respectively. Temperature-sensitive (ts) mutants of these strains, LA334 (of B77), LA31 (of PR-RSV) or OS122 (of SR-RSV), showed similar changes at 36°C. At 41°C, files of the membrane proteins were similar to those of uninfected cells; this was true except in the case of the decrease of the 200,000 dalton protein. The appearance of the 90,000 molecular wt polypeptide and the decrease in the 50,000 dalton polypeptide were reversible and clearly observable within a few hours after a temperature shift of CEF infected with ts mutants. Fusiform transformation induced by a variant of B77 was also shown to induce these two alterations. From these and other results, the appearance of the polypeptide band of 90,000 daltons which could not be detected in untransformed cells, and the marked decrease in amount of a protein of 50,000 daltons in cell membranes were concluded to be closely correlated with transformation of CEF.

226. ALTERATIONS IN SURFACE PROTEINS IN CHICKEN CELLS TRANSFORMED BY TEMPERATURE-SENSITIVE MUTANTS OF ROUS SARCOMA VIRUS.

Hynes RO, Wyke A
Imp. Cancer Res. Fund Lab., London, England
Virology; 64(2):492-504 1975

Temperature-sensitive (ts) mutants of Rous sarcoma virus (RSV) and lactoperoxidase-catalyzed iodination were used to study the changes in surface proteins associated with viral transformation. Secondary chick embryo fibroblast (CEF) cultures were infected with mutants of the Prague strain of RSV, subgroup A, and were incubated at the permissive temperature (35°C) until the first clear signs of cell transformation at 3-4 days postinfection. The cultures were

then maintained until the seventh day postinfection at the nonpermissive temperature (41°C), after which they were transferred to Nunc plastic petri dishes and maintained for 36-48 hr at 35 or 41°C. All cell monolayers were incubated with (125I)iodide, lactoperoxidase and glucose oxidase for 10 min at room temperature. The cultures were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and determinations were made of the rate of 2-deoxy-D-glucose uptake and the rate of protein synthesis after incubation with (35S)methionine. A large external transformation-sensitive (LETS) protein was detected by lactoperoxidase-catalyzed iodination on normal CEF but was reduced on the RSV-transformed cells. The LETS protein was temperature sensitive in the ts RSV-infected CEF: during reversion to normality on temperature shift-up, the protein appeared at the surface quite rapidly and in parallel with the morphological changes due to transformation, although the change was less rapid than that of glucose transport rate; on transformation during temperature shift-down, the disappearance of the LETS protein lagged behind the morphological change. Cycloheximide did not inhibit the reappearance of the iodinated LETS protein on shift-up, which suggests that the protein is synthesized in the transformed cells although it is not present at the surface. The data suggest an increased rate of turnover of the LETS protein after transformation, possibly due to the involvement of proteases released by the transformation process.

227. POLYPEPTIDE COMPOSITION OF CELL MEMBRANES FROM CHICK EMBRYO FIBROBLASTS TRANSFORMED BY ROUS SARCOMA VIRUS.

Marciani DJ, Bader JP

Natl. Cancer Inst., Bethesda, MD 20014

Biochim Biophys Acta; 401(3):386-398 1975

Chick embryo fibroblasts were transformed by the Bryan high-titer strain of Rous sarcoma virus (RSV-BH), or a mutant (RSV-BH-Ta) inducing temperature-dependent transformation. Surface membranes from normal and transformed cells were isolated as membrane vesicles by differential centrifugation and as cell ghosts after ZnCl₂ treatment and separation in an aqueous two-phase system. These preparations were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate or phenol/urea/acetic acid. In general a greater resolution of individual bands was found in gels containing phenol/urea/acetic acid, which separates polypeptides on the bases of size and charge. Electrophoresis of preparations from nontransformed cells showed that two polypeptides (molecular wt 200,000 and 250,000) found in cell ghosts were missing in membrane vesicles. In cell ghosts, transformation by RSV-BH resulted in a significant decrease of the 250,000 molecular wt complex. Also a polypeptide (molecular wt 73,000) prominent in membrane vesicles from nontransformed cells was decreased in transformed cells. Surfaces from cells transformed by RSV-BH-Ta at 37°C presented patterns similar to those for RSV-BH infected cells. Shifting these cells to 41°C resulted in an increase in the 250,000 molecular wt complex, although the amount of this protein(s) never reached that found in noninfected cells. Inhibitors of RNA and protein synthesis (actinomycin D and cycloheximide, resp) failed to block the morphological changes occurring in RSV-BH-Ta cells after temperature shifts from 41°C to 37°C or vice versa. The same inhibitors caused a reduction in the levels of the 250,000 molecular wt complex at both temperatures. These data indicate that these large membrane-associated polypeptides play little or no role in the morphological changes associated with transformation and its reversal.

228. A CONSIDERATION OF THE ROLE OF CELL SURFACE MACROMOLECULES IN THE PROCESS OF VIRAL TRANSFORMATION.

Sheinin R, Shopsis C

Ontario Cancer Inst., Univ. Toronto, Toronto, Canada

M4X 1K9

In Vitro; 12(3):198-207 1976

An isogenic mutant of simian virus 40 (SV40)-transformed 3T3 mouse fibroblasts that is temperature-sensitive in its growth properties (ts H6-15) was used to study biochemical modification of the cell surface during viral transformation. A comparison was made of several plasma membrane constituents of ts H6-15 cells grown at permissive (32°C) and nonpermissive (39°C) temperatures. (At 32°C, they resembled the wild-type virus-transformed cells, and at 39°C, they behaved more like untransformed 3T3 mouse fibroblasts.) Since the pattern of amino acid labeling of plasma membrane polypeptides was essentially the same for ts H6-15 cells incubated at 32 and 39°C, the pattern of synthesis of most of the polypeptides is not a key determinant of transformed cell growth. With the use of lactoperoxidase-catalyzed iodination (¹²⁵I), a trypsin-sensitive, surface-exposed, 240,000-molecular-wt protein was detected in approximately equal amounts in cells grown at both temperatures. Proteins with a molecular wt of 120,000, 50,000, and 42,000 were detected in much greater amounts in cells grown at 39°C. When comparisons were made of glycoproteins using a glucosamine (Glc-NH₂) label, more extensive labeling of a number of high-molecular-weight (about 100,000-160,000) glycopeptides was observed at 32 than at 39°C. Cells that grew in the pattern of transformed cells also appeared to make one glycoprotein of 120,000 molecular wt that was barely detectable in cells grown at 39°C. Upon examination of surface-exposed glycoproteins using Glc-NH₂ and enzyme-catalyzed labeling, no significant qualitative differences were found at the two temperatures. In addition, no differences in the pattern of glycosphingolipid synthesis were detected, and it was concluded that changes in this synthesis are not essential for transformed cell growth. Data suggest that alterations in the metabolism of the high-molecular-weight surface glycoprotein may be a secondary phenomenon of transformation associated with specific cells and/or specific viruses. (52 refs)

229. A STRUCTURAL CHANGE OF THE PLASMA MEMBRANE INDUCED BY ONCOGENIC VIRUSES: QUANTITATIVE STUDIES WITH THE FREEZE-FRACTURE TECHNIQUE. (MEETING ABSTRACT).

Torrier G, Montagnier L, Biquard JM, Vigier P

Département de Virologie, Institut Pasteur 25, rue du Dr Roux, 75015 Paris, France

Proc Natl Acad Sci USA; 72(5):1695-1698 1975

The density of intramembranous particles (IM particles) were studied quantitatively by a freeze-etching technique in mammalian and a rat cell systems. Two clones were derived from the asparagine-dependent subclone C13/8 from BHK21/13 line hamster fibroblasts: (1) C13/8/HS5, transformed by hamster sarcoma virus, and (2) C13/8/SPy2, transformed by polyoma virus. A mutant of Schmidt-Ruppin strain of Rous sarcoma virus (RAV 1) was used for infecting and transforming secondary cultures of chick embryo fibroblasts (CEF). Hamster cells and CEF cells were harvested for freeze-fracturing. Thirty micrographs of each cell preparation were selected at random, and counts of 1 microm² of each picture were statistically analyzed. No deviation from random distribution of IM particles was observed in the membrane fractures of normal or virus transformed BHK21 cells. However, three times more particles were observed in the clone transformed by hamster sarcoma virus than in control cells. CEF infected with Rous-associated virus type 1 (RAV 1)

also demonstrated a significant increase in particle density. The increase in particles is either related to virus-induced transformation, or to virus production only. The authors suggest that the primary change induced by the virus may be due to the insertion of new proteins in the hydrophobic portion of the plasma membrane.

230. SURFACE RUFFLES AS MARKERS FOR STUDIES OF CELL TRANSFORMATION BY ROUS SARCOMA VIRUS (MEETING ABSTRACT).

Ambros VR, Bo-Chen L, Buchanan JM

Dept. of Biology, Massachusetts Inst. of Technology, Cambridge, MA 02139

Proc Natl Acad Sci USA: 72(8):3144-3148 1975

Confluent chick embryo fibroblasts infected with the Ts68 mutant of Rous sarcoma virus were examined by scanning electron microscopy at the permissive (36°C) and nonpermissive (41°C) temperatures for transformation. Infected cells (but not mock-infected cells) shifted from 41 to 36°C and changed from elongated to rounded. This was preceded by the appearance of surface ruffles on the cell. These surface ruffles were not observed on cells maintained at 41°C, and appeared as early as 0.5 hr after a shift to 36°C. After two hr, more than 70% of the cells had ruffles. By 3.5 hr after the shift from 41 to 36°C, cultures were fully transformed by the criteria of cell roundedness and the presence of surface ruffles. This surface ruffling of cells is the earliest event so far reported during the transformation process and is not dependent upon protein synthesis as indicated by the lack of effect of cycloheximide on rounding or ruffling of the cells. It appears that a temperature-sensitive initiator of surface changes exists in the infected cells held at 41°C.

231. PLASMA MEMBRANE ALTERATION ASSOCIATED WITH MALIGNANT TRANSFORMATION IN CULTURE.

Gilula NB, Eger RR, Rifkin DB

The Rockefeller University, New York, NY 10021

Proc Natl Acad Sci: 72(9):3594-3598 1975

The intramembrane organization of the plasma membranes of nonmalignant cells in culture has been compared by freeze-fracturing with that of virally-transformed malignant cells. No dramatic differences are present in the distribution of intramembrane particles in the plasma membranes of these cells when the cells are examined without fixation or with mild fixation (glutaraldehyde treatment) prior to freezing. However, a redistribution of intramembrane particles into aggregates occurs in the membranes of nontransformed cells after treatment with glycerol. The aggregation of particles is extensive in normal chick embryo fibroblasts, and less extensive in mouse 3T3 cells. The glycerol-induced particle redistribution is not inhibited at 4 degrees, but it is inhibited by pretreatment with 2.5% glutaraldehyde. A significant number of the cells remain viable after the glycerol treatment, and the process is reversible. Particle aggregate does not appear to be related to either growth rate or cell density. Transformed Rous sarcoma virus/chick embryo fibroblasts and simian virus 40/3T3 cells have few particle aggregates after glycerol treatment. The plasma membranes of chick embryo fibroblasts transformed with a mutant of Rous sarcoma virus (TS-68) that is temperature sensitive for transformation, have few particle aggregates when grown at the permissive temperature (37 degrees). Extremely prominent particle aggregates are present in the plasma membranes of cells grown at the nonpermissive temperature (41 degrees). These observations indicate that there is an alteration in the plasma membrane associated with viral transformation which is related to a glycerol-sensitive mechanism that controls the distribution of intramembrane particles. (Author Abstract)

232. SUBSTRATE-ATTACHES GLYCOPROTEINS MEDIATING ADHESION OF NORMAL AND VIRUS-TRANSFORMED MOUSE FIBROBLASTS.

Culp LA

Sch. Med., Case Western Reserve U., Cleveland, OH
J Cell Biol; 63:71-83 1974

When BALB/c 3T3, simian virus 40 (SV40)-transformed 3T3 (SVT2), and revertant variants of the transformed cells are removed by sodium edetate treatment from the substrate on which they are grown, they leave behind a glycoprotein layer of substrate-attached material (SAM). All three cell types displayed a 30% higher plating efficiency when grown on 3T3 SAM. SVT2 cells grown on 3T3 SAM were comparatively more spread out on the substrate, resisted crawling over neighboring cells, and resisted movement away from the edges of colonies; SVT2 SAM did not cause these changes. SVT2 cells attached to 3T3 SAM- or SVT2 SAM-coated cover slips with a faster initial rate and to a higher saturation level than to untreated substrate; 3T3 and revertant cells exhibited no preference. There was no species specificity in these phenomena. Trypsin-released cells attached much more slowly than sodium edetate-released cells. 3T3 SAM was not effective in lowering the saturation density of mass cultures of virus-transformed cells. The data suggest that the substrate-attached glycoproteins of normal cells affect the cellular adhesivity, morphology, movement, and perhaps growth patterns of virus-transformed cells, causing partial reversion of these properties of transformed cells to those found in contact-inhibited fibroblasts.

233. METABOLIC PROPERTIES OF SUBSTRATE-ATTACHED GLYCOPROTEINS FROM NORMAL AND VIRUS-TRANSFORMED CELLS.

Culp LA, Terry AH, Buniel JF

Case West. Reserve Univ. Sch. Med., Cleveland, OH
Biochemistry (Washington): 14(2):406-412 1975

BALB/c 3T3, SV40-transformed 3T3 cells, and concanavalin A revertant variants of transformed cells leave a layer of glycoprotein on the culture substrate upon ethylenediamine (oxyethylenetriple)tetraacetic acid-mediated removal of cells. The metabolic properties of this glycoprotein have been studied, including its accumulation on the substrate, rate of synthesis, and turnover as a function of growth of contact-inhibited and virus-transformed cells. Pulse and cumulative radiolabeling experiments with glucosamine and leucine precursors established that this glycoprotein accumulates on the substrate in growing cultures until cells have completely covered the substrate. The synthesis and/or deposition of the material diminished dramatically in cultures whose substrates had been completely covered with cells, even though the contact-inhibited cell lines continued to make cell-associated and medium secreted glycoproteins, and transformed cells continued to divide and form multilayered cultures. Pulse-chase analysis using long periods of pulsing with radioactive leucine showed that these glycoproteins are deposited directly on the substrate by cells and not subsequent to secretion into the medium. The glycoprotein that accumulated during long pulses was stably adherent to the substrate and displayed little appreciable turnover during three days of chasing of either sparse or dense cultures. Short-term pulse-chase analysis with leucine revealed two metabolically different pools of material: one which turns over very rapidly with a half-life of two to three hr and a second pool which is stably deposited on the substrate and whose proportion increased with the length of the radiolabeling period. It is concluded that there are no appreciable differences in the metabolic properties of the glycoprotein observed in the three cell types studied during growth on a plastic substrate. These results are discussed with regard to the implicated roles of these glycoproteins in mediating

adhesion of normal and virus-transformed cells to the substrate.

234. SUBSTRATE-ATTACHED SERUM AND CELL PROTEINS IN ADHESION OF MOUSE FIBROBLASTS.

Culp LA, Buniel JF

Sch. Medicine, Case Western Reserve Univ., Cleveland, OH 44106

J Cell Physiol; 88(1):89-106 1976

The effects of serum and coatings of substrate-attached material (SAM) on the kinetics of attachment of DNA-radiolabeled BALB/c 3T3, SV40-transformed 3T3, and concanavalin A-selected revertant cells to glass coverslips were studied. The presence of serum in the medium of attaching cells had a marked effect on the initial time lag before stable attachment of cells, the maximum level of attached cells, the stability of attachment, and the pseudopodial spread of the cell over the substrate. These serum effects could be mimicked by measuring attachment in medium without serum and with use of serum-preadsorbed or 3T3 SAM-coated coverslips. Enzymatic treatment of serum-preadsorbed substrates indicated that the factor(s) in serum that affects attachment was very trypsin-sensitive. Serum preadsorption of substrates stimulated attachment of SVT2 cells in medium with serum in a manner very similar to the effects of 3T3 SAM coatings, while attachment of 3T3 or revertant cells was unaffected. Slab gel electrophoretic analysis identified eight major serum proteins (Coomassie blue staining) that bind to the substrate in the absence of cells and persist on the substrate after growth to confluence of 3T3 or SVT2 cells; this suggests that major breakdown of serum-adsorbed components does not occur during growth of normal or transformed cells. Seven radioactive SAM proteins were detected by autoradiography in 3T3 or SVT2 SAM electropherograms, two of which were high molecular wt components corresponding to the glucosamine-radiolabeled hyaluronate proteoglycans observed previously; the remaining five are newly identified proteins in SAM. 3T3 and SVT2 cells have unique proportions of these seven components. It is not known which of the eight serum proteins and seven cell-synthesized SAM components are important in substrate adhesion or what temporal and spatial interactions occur among the cell surface, the serum components adsorbed to the substrate and the substrate-attached material in terms of adhesion to and movement on an artificial substrate. The data are consistent with the idea that normal or virus-transformed cells do not attach directly to the culture substrate, but to specific classes of substrate-adsorbed serum proteins via deposition of specific classes of cell surface proteins and polysaccharides.

235. EARLY EVENTS DURING SUBSTRATE ADHESION OF NORMAL AND VIRUS-TRANSFORMED MOUSE FIBROBLASTS.

Mapstone TB, Culp LA

Sch. Medicine, Case Western Reserve Univ., Cleveland, OH 44106

J Cell Sci; 20(3):479-495 1976

The relationship between attachment of BALB/c 3T3 cells and their Simian virus 40 (SV40) transformants (SVT2) to glass of plastic substrates and deposition of substrate-attached material (SAM - proteoglycans implicated in substrate adhesion) was examined very early after inoculation of cells subcultured with ethylenbis(oxyethylenitrilo)tetraacetic acid (EGTA). Cycloheximide and colchicine minimally affected the kinetics or short-term stability of attachment of cells or deposition of SAM. SAM deposition onto the substrate began immediately after inoculation of cells and was maximal prior to the highest cell attachment level (30-40 min after inoculation). At 4°C, there was no attachment of cells to the substrate and no deposition of leucine- or glucosamine-

radiolabeled SAM on to the substrate. 3T3 cells deposited SAM to a maximal level earlier during the attachment process than SV40-transformed cells. The SVT2 cells deposited much smaller amounts of SAM (measured on a per-cell basis) to 3T3 SAM-coated substrates during attachment processes, whereas 3T3 cells and concanavalin A (con A) revertant variants of SVT2 cells, which have regained density-dependent inhibition of growth, deposited identical amounts of SAM (per-cell) on untreated or SAM-coated substrates. Serial attachment experiments with SVT2 cells indicated that all SVT2 cells reduced their deposition amounts on SAM-coated substrates, rather than there being an ability of a small proportion of cells to attach preferentially to SAM-coated substrates while being unable to deposit SAM themselves. The data are consistent with the presence of a sizeable pool of SAM-like proteoglycans being present on the surface of EGTA-removed cells whose deposition may be a requirement for, but may not necessarily be sufficient for, stable adhesion cells to the substrate.

236. MUTANTS OF BALB/C3T3 FIBROBLASTS DEFECTIVE IN ADHESIVENESS TO SUBSTRATUM: EVIDENCE FOR ALTERATION IN CELL SURFACE PROTEINS (MEETING ABSTRACT).

Pouyssegur JM, Pastan I

Lab. Molecular Biology, Natl. Cancer Inst., Natl. Insts. Health, Bethesda, MD 20014

Proc Natl Acad Sci USA; 73(2):544-548 1976

Two mutants of BALB/c 3T3 cells defective in adhesiveness to substratum were studied to investigate cell growth regulation mechanisms. After exposure to a mutagen a cell population was treated with prostaglandin E1 and methylisobutylxanthine to elevate the cells 3',5'-cyclic AMP levels and adhesion to the substratum; then loosely adherent cells were harvested. After four cycles of selection two clones were isolated. Both were characterized by a round morphology and a decreased adhesion to substratum. The defect in adhesion appeared to be responsible for the change in cell shape, since the mutant cells were flattened and looked similar to wild-type cells when treated with dibutyryl cyclic AMP (though adhesiveness remains decreased). Lactoperoxidase-catalyzed iodination of the cell surface showed that two iodinated polypeptides (molecular wt, Mr, 90,000 and 135-140,000) present in wild-type cells not detected on the surface of the mutants, and another iodinated band (110-120,000 Mr) markedly decreased. Also, one mutant was missing the high-molecular-wt (230,000) cell surface protein that is sensitive to transformation. No changes were found in the content of cyclic AMP or free or polymerized tubulin. The exponential growth rate and saturation density was not altered in these adhesion-deficient cells. This last result suggests that modulation of adhesion to substratum per se does not play an important role in the growth control of Balb/c3T3 cells in vitro.

237. CELL SURFACE PROTEIN PARTIALLY RESTORES MORPHOLOGY, ADHESIVENESS, AND CONTACT INHIBITION OF MOVEMENT TO TRANSFORMED FIBROBLASTS (MEETING ABSTRACT).

Yamada KM, Yamada SS, Pastan I

Natl. Cancer Inst., Natl. Insts. Health, Bethesda, MD 20014

Proc Natl Acad Sci USA; 73(4):1217-1221 1976

The major cell surface glycoprotein of chick embryo fibroblasts, CSP, was isolated and added to a variety of transformed cells in vitro. The transformed cells became more elongated, often more flattened, and showed increased adhesion to the substratum. Several transformed cell lines also aligned in striking parallel arrays. This alignment was characterized by a decrease in the amount of nuclear overlapping, probably indicating restoration of contact inhibition of

movement. The morphological changes were antagonized by antibody to CSP. These effects of CSP were associated with an elevation of cellular 39:59-cyclic AMP. Moreover, the morphological reversion was not accompanied by an alteration in growth properties. These results are consistent with a role for CSP in cell adhesion and morphology but not in growth control.

238. DECREASED ADHERENCE TO THE SUBSTRATE IN ROUS SARCOMA VIRUS-TRANSFORMED CHICKEN EMBRYO FIBROBLASTS.

Weber MJ, Hale AH, Losasso L
Department of Microbiology, University of Illinois
Urbana, IL 61801
Cell (Camb); 10(1):45-51 1977

Cell-substrate adherence in cultures of chicken embryo fibroblasts was examined by determining the number of cells which could be detached from the culture dish by a stream of medium. Transformed cells were significantly less adherent than their normal counterparts. In cultures infected with a mutant of Rous sarcoma virus which is temperature-conditional for transformation, adherence changed promptly following a temperature shift. This change did not require progression through the cell cycle. The transformation-specific decrease in adherence required new protein synthesis, but the restoration of adherence which occurred following a shift to the restrictive temperature could occur in the absence of new protein synthesis. Inhibitor experiments suggested the importance of microfilaments and perhaps microtubules in the changes in detachability. In addition, there was a positive correlation between levels of surface LETS protein and cell substrate adherence following a temperature shift, although it seems probable that the bulk of the surface LETS is neither necessary nor sufficient for maintenance of normal cell substrate adherence. (Author Abstract)

239. A COMMON BIOCHEMICAL CHANGE IN SV40 AND POLYOMA VIRUS-TRANSFORMED MOUSE CELLS COUPLED TO CONTROL OF CELL GROWTH IN CULTURE.

Mora PT, Cumar FA, Brady R
Natl. Inst. Neur. Dis. Stroke, Natl. Inst. Hlth., Bethesda, MD
Virology; 46(1):60-72 1971

An investigation is presented which deals with enzyme depression in virally infected permissive cells, biochemical changes in transformed cells, and enzyme activity in cocultivation of transformed and nonvirally transformed cells. Cell lines, cloned derivatives from a single cell, from mouse embryos were used as well as 3T3 cells. Loss of specific activity of amino sugar transferase did not occur after productive infection by polyoma virus in the two lines studied. The effect of the virus in altering the growth properties of the cells and the biosynthesis of the higher gangliosides appear to be closely coupled. In polyoma and SV40 virus transformed cell lines, the higher gangliosides were greatly reduced, concomitant with the reduction of the activity of the hematoside N-acetylglucosaminyltransferase. Changes in the amino sugar transferase activity paralleled the changes in phenotypic growth properties in cultures of both polyoma and SV40 virus-transformed cells and in the flat derivatives of these lines where there was a trend toward reversion to levels in uninfected cells. In cocultivation experiments no evidence of a factor influencing enzyme activity was detected in either the enzymatically active or inactive cells. Enzyme inhibitor was not detectable in the homogenate or in subcellular fractions of the virally transformed cells. It is assumed that the control of enzyme activity occurs at the protein biosynthesis level. The repressor-like mechanism in SV40 and polyoma virus induced cell transformation is paralleled by the biochemical findings.

240. SURFACE GLYCOPROTEINS AND GLYCOLIPIDS OF CHICKEN EMBRYO CELLS TRANSFORMED BY A TEMPERATURE-SENSITIVE MUTANT OF ROUS SARCOMA VIRUS.

Warren L, Critchley D, MacPherson I
Imperial Cancer Res. Fund Lab., London, England
Nature (Lond); 235(5336):275-278 1972

Surface glycoproteins and glycolipids were analyzed in chicken embryo cells transformed by a temperature-sensitive Rous sarcoma virus (RSV) strain T5. Chicken embryo cells transformed by T5 are morphologically like cells transformed by wild type RSV(SR) at 35-36 C but lose their transformed morphology at 41 C. A dual label technique was used to compare surface glycoproteins of normal and T5-transformed cells. 14C-Fucose was added to normal cell cultures and 3H-fucose was added to transformed cultures one day after cells were shifted to 35 C or 42 C. The cells were trypsinized 72 hr after addition of isotope before they became confluent. Pronase-digested 'trypsinates' of 14C-labeled control cells and 3H-labeled RSV(SR)- or T5-transformed cells were mixed and chromatographed together on Sephadex G-50. Cell surface material from cells transformed by T5 and grown at 35 C displayed an elution pattern similar to that seen with cell surface material from wild type RSV(SR)-transformed cells grown at either 35 C or 41 C. The larger surface material characteristic of RSV(SR)-transformed cells and T5-transformed cells grown at 35 C disappeared when T5 cells were shifted to 41 C. Similar results were obtained when RAV 1-transformed cell surfaces were analyzed, indicating that the presence of carbohydrate-containing proteins of larger size on the surface of cells is associated with transformation rather than with a particular virus infection. Normal and transformed cells labeled with 14C-palmitate were also analyzed for their total neutral lipids, phospholipids and glycolipids by thin-layer chromatography. No marked difference in incorporation of isotope into glycolipids of normal and RSV(SR)-transformed cells was found at either 35 C or 41 C. Ceramide monohexoside and hematoside were the major glycolipids present. The results thus confirm previous reports that the surface of virus-transformed cells have relatively large amounts of fucose-containing glycoproteins. The mechanism of increase is unknown.

241. THE ISOLATION AND CHARACTERIZATION OF PLASMA MEMBRANE FROM CULTURED CELLS. IV. THE CARBOHYDRATE COMPOSITION OF MEMBRANES ISOLATED FROM ONCOGENIC RNA VIRUS-CONVERTED CHICK EMBRYO FIBROBLASTS.

Perdue JF, Kletzien R, Wray VL
McArdle Lab. Cancer Res., U. Wisconsin, Madison, WI
Biochim Biophys Acta; 266(2):505-510 1972

Cell membrane fractions were isolated by density gradient centrifugation of homogenates of cultured chick embryo fibroblasts converted by avian sarcoma viruses, morpho Fujinami virus and a mutant of this virus, morpho Fujinami virus. Membrane fractions were concentrated in sucrose at a density of 1.058 plus or minus 0.001 (A' band of morpho-converted cells) and 1.074 plus or minus 0.004 (A' band of morpho-converted cells). The membranes in the B' band from cells converted by either virus were both concentrated at density of 1.11. Estimates of sialic acid content in the membrane fractions were 75 nmoles/mg protein in the A' band from morpho-converted cells and 64 nmoles/mg protein in the A' band from morpho-converted cells. Sialic acid levels were 50% higher in uninfected cells. Neutral sugar levels in the A' and B' band membranes from morpho-converted cells were 235 and 152 microg/mg protein, the same as those recently reported for uninfected cells. The morpho-converted cells had 40% more neutral sugar (331 microg/mg protein) in the A' band

than did uninfected cells. This value was similar to that reported for RBA-virus-transformed cells. An analysis of the amino sugar content (0.13-0.19 microg./moles/mg protein) of the cell membranes in the A' and B' bands from uninfected fibroblasts and fibroblasts infected with leukemia virus (RAV-49), or infected and converted by sarcoma virus RBA and morphf and morphr Fujinami, did not establish compositional differences which could be related to morphological cell shape or virus conversion. The most consistent finding was the decrease in sialic acid content of membranes from the cells converted by the oncogenic viruses (RBA, morphf and morphr Fujinami). Since this decrease also did not correlate with cell shape and was not observed in leukemia virus-infected cells, it represents a fundamental alteration in membrane chemistry paralleling a loss of the fibroblasts' controls of growth and multiplication.

242. SURFACE GLYCOPROTEINS OF CELLS BEFORE AND AFTER TRANSFORMATION BY ONCOGENIC VIRUSES.

Warren L., Fuhrer JP, Buck CA

Sch. Med., U. Pennsylvania, Philadelphia, PA

Fed Proc; 32(1):80-85 1973

The double label method employing L-fucose -14C and -3H was used to compare the carbohydrate components of the surface glycoproteins of control and virus-transformed cells. A material eluting early from a column of Sephadex G-50 was found in relatively large quantity in transformed cells compared with controls. The cells must be in a growing state for this peak to appear. This material elutes early because it contains extra residue of sialic acid. A sialyl transferase was found that may account for the formation of this material. Its activity is 2.5- to 11-fold greater in transformed cells than in controls and activity decreases when the cells are not dividing. Work with chick embryo fibroblasts transformed by TS, a temperature-sensitive mutant of Rous sarcoma virus, shows that the peak of early eluting material and the sialyl transferase activity are present in considerably greater amounts of the permissive temperature of 36 C where transformation is manifested than at 41 C where the cells appear and behave as if normal.

243. SURFACE MEMBRANE GLYCOPEPTIDES CORRELATED WITH TUMORIGENESIS.

Glick MC, Rabinowitz Z, Sacks L

Weizmann Inst. Sci., Rehovot, Israel

Biochemistry (Washington); 12(24):4864-4869 1973

Glycopeptides were removed by trypsin from the surface membranes of the following cell types: hamster embryo cells transformed after treatment with the chemical carcinogen, dimethylnitrosamine; variants of these cells with suppression of the malignant transformed properties; and revertants of these variant cells to the transformed state. The glycopeptides were examined by gel filtration after Pronase digestion. In all cases, the gel filtration profiles of the fucose-containing glycopeptides were similar to those obtained from secondary hamster embryo cells. In contrast, profiles derived from tumors formed after inoculation into animals of all of these cell lines had the appearance of a specific group of glycopeptides not found in the original cells. Other properties characteristic of the transformed phenotype, such as ability to overcome contact inhibition and to form colonies with high efficiency in soft agar and liquid medium, failed to show a consistent correlation either with the nature of the surface glycopeptides or with the ability to produce tumors. These results demonstrate a change in the surface membrane glycopeptides with tumor formation and suggest a correlation between the two.

244. CHANGES IN A SURFACE-LABELLED GALACTO-PROTEIN AND IN GLYCOLIPID CONCENTRATIONS IN CELLS TRANSFORMED BY A TEMPERATURE-SENSITIVE POLYOMA VIRUS MUTANT.

Gahmberg CG, Kiehn D, Hakomori SI

Dept. Pathobiol., U. Washington, Seattle, WA

Nature (Lond); 248(5447):413-415 1974

BHK cells transformed with temperature-sensitive polyoma virus (BHKpyts3 Cl 7C), those transformed with wild type polyoma virus (BHKpywt Cl 4), and their progenitory cells (BHK Cl 13) were surface labeled. A surface galactoprotein of BHK with a molecular weight of 200,000 (galactoprotein a) was not labeled in BHKpywt at 32 or 39 C, or in BHKpyts3 at 32 C; it was labeled in BHKpyts3 grown at 39 C. In actively growing, sparse or subconfluent BHK cells, galactoprotein a was not labeled, whereas it was labeled when growth was inhibited at the saturation density. In BHKpywt, the chemical concentration of lactosylceramide was higher than in normal BHK cells, and the concentration of trihexosylceramide was considerably reduced. The concentration of lactosylceramide was also sharply increased at 39 C, whereas the concentration of hematoside was depressed. The trihexosylceramide concentration in BHKpyts3 was low at both temperatures. The results suggest that although trihexosylceramide reduction accompanies polyoma virus transformation, it is not under the control of the ts3 gene function and is not implicated in the regulation of cell growth. Increased lactosylceramide could be essential for the expression of crucial aspects of the transformed phenotype, including the regulation of growth. The galactoprotein a, lactosylceramide, and hematoside concentrations may be related to growth control in BHK cells.

245. SURFACE BIOCHEMICAL CHANGES ACCOMPANYING PRIMARY INFECTION WITH ROUS SARCOMA VIRUS. I. ELECTROKINETIC PROPERTIES OF CELLS AND CELL SURFACE GLYCOPROTEIN: GLYCOSYL TRANSFERASE ACTIVITIES.

Bosmann HB, Case KR, Morgan HR

U. Rochester, Sch. Med. Dent., NY

Exp Cell Res; 83(1):15-24 1974

Both chick embryo fibroblasts (CEF) and CEF transformed by the Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV) contain glycosyl ectoenzyme systems. Both the glycoprotein:glycosyl transferase and acceptors appear to be located on the external plasma membrane of both cell lines. The activity of the uridine diphosphate (UDP)-galactose and guanosine diphosphate (GDP)-mannose ectoenzyme systems was particularly high. Compared with the normal CEF, the activities of the UDP-galactose and GDP-mannose glycosyl ectoenzyme systems were elevated 12-fold. CEF infected with leukemia virus (RAV) or a temperature-sensitive RSV mutant (TS-68) at the nonpermissive temperature had glycosyl ectoenzyme levels similar to those of the normal CEF, suggesting that the elevations in the SR-RSV-infected cells were the result of transformation rather than merely viral infection. These glycosyl transferases and acceptors with activities expressible at the cell surface may be important in cell:cell adhesion and related phenomena or in the glycosylation of extracellular glycoproteins. SR-RSV CEF in 0.0145 M NaCl, 0.6mM NaHCO₃, 4.5% sorbitol, pH 7.2, at 25 C had an electrophoretic mobility of -2.45 plus or minus 0.02 microm/s/V/cm, while control CEF had a mobility of -2.04 plus or minus 0.01 microm/s/V/cm. RAV-CEF grown at 37 C and TS-68 CEF grown at 41 C had mobilities of -2.03 and -2.02 microm/s/V/cm, respectively, indicating that the elevated electrophoretic mobility shown by the SR-RSV CEF was probably the result of viral transformation. The elevation in the electrophoretic mobility of the SR-RSV CEF was due

primarily to elevated levels of surface N-acetylneuraminic acid and hyaluronic acid.

246. SURFACE BIOCHEMICAL CHANGES ACCOMPANYING PRIMARY INFECTION WITH ROUS SARCOMA VIRUS. II. PROTEOLYTIC AND GLYCOSIDASE ACTIVITY AND SUBLETHAL AUTOLYSIS.

Bosmann HB, Lockwood T, Morgan HR
U. Rochester, Sch. Med., Dent., NY
Exp Cell Res; 83(1):25-30 1974

Normal chick embryo fibroblasts (CEF) were found to have low levels (less than 10 nmoles/106 cells/hr) of beta-fucosidase and alpha-mannosidase activity, intermediate levels (less than 50 nmoles/106 cells/hr) of N-acetyl-beta-galactosaminidase and beta-galactosidase, and high levels (less than 100 nmoles/106 cells/hr) of N-acetyl-beta-glucosaminidase and acid phosphatase. High levels of pH 3.4 protease and low levels of pH 7.4 protease were also found. CEF infected and transformed with the Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV) had elevated levels of beta-galactosidase, beta-fucosidase, N-acetylglucosaminidase, N-acetyl-galactosaminidase, and alpha-mannosidase activity; the acid phosphatase activity was not elevated. Proteolytic activity at pH 3.4 (cathepsin-like activity) was greatly elevated in the SR-RSV-CEF, while proteolytic activity at pH 7.4 (trypsin-like activity) was slightly lower than in normal CEF. Elevated levels of these degradative enzymes were not found in cells infected with leukemia virus (RAV-1). Similarly, with a temperature-sensitive mutant of SR-RSV (TS-68), the elevations in the activity of the degradative enzymes occurred only at the permissive temperature. The observed elevations in the activities of the degradative enzymes may function for sublethal autolysis or the maintenance of the neoplastic state.

247. CELL SURFACE CHANGES CORRELATED WITH DENSITY-DEPENDENT GROWTH INHIBITION. GLYCOSAMINOGLYCAN METABOLISM IN 3T3, SV3T3, AND CON A SELECTED REVERTANT CELLS

Roblin R, Albert SO, Gelb NA, Black PH
Harvard Med. Sch., Boston, MA
Biochemistry (Washington); 14(2):347-357 1975

A ³⁵SO₄ labeling/chromatography technique has been developed which facilitates quantitation of sulfated glycosaminoglycan (GAG) synthesis in mammalian cell cultures. The technique has been used to compare sulfated GAG biosynthesis, degradation, and turnover in three related cell lines with differing degrees of density-dependent growth inhibition in vitro: BALB/c 3T3, (SV3T3), and SV3T3 revertant cells. SV3T3 cells incorporated 2- to 5-fold less ³⁵SO₄ than their untransformed counterparts. SV3T3 revertant cells, which show partial reversion to low saturation density in vitro, showed a 2.5- to 8-fold increase in cell-associated sulfated GAG compared with the parental SV3T3 cells from which they were selected. Mild trypsin treatment of ³⁵SO₄-labeled cells removed 68-84% of the cellular sulfated GAG, suggesting that at least this proportion of the total cellular sulfated GAG was located at the cell periphery. Removal of ³⁵SO₄-labeled cells from the Petri dish with a Ca++ selective chelating agent revealed a fraction of GAG that remained tightly bound to the dish. A higher proportion of the total cell-associated sulfated GAG remained attached to the Petri dish in untransformed and revertant cell cultures compared with that present in transformed cell cultures. It is suggested that a correlation exists between a relatively high level of GAG synthesis and low saturation density in cell cultures. CITATION DELIMITER

248. FLUORESCENCE POLARIZATION AND VISCOSITIES OF MEMBRANE LIPIDS OF 3T3 CELLS (MEETING ABSTRACT).

Fuchs P, Parola A, Robbins PW, Blout ER
Center for Cancer Res., Massachusetts Inst. Technology,
Cambridge, MA 02139
Proc Natl Acad Sci USA; 72(9):3351-3354 1975

Fluorescence polarization and microviscosity values of the lipid bilayer of membranes of normal, transformed and revertant 3T3 cells were obtained using a fluorescent hydrocarbon, 1,6-diphenyl-1,3,5-hexatriene. Following 60 min of labeling, fluorescence polarization values of normal cells were significantly lower than those of cells transformed with either polyoma virus or the Simian virus 40. Fluorescence polarization values of Py6R1 cells, a revertant line isolated from 3T3Py6 cells by a fluorodeoxyuridine procedure, were also lower than those obtained with normal cells. Microviscosity values, calculated from mean fluorescence anisotropy values were significantly higher in transformed cells and revealed a 50% increase. Possible correlations between the bilayer viscosity and the mobility of the receptors in the membrane are discussed.

249. MEMBRANE LIPID ACYL GROUP ALTERATIONS IN CELLS INFECTED WITH A TEMPERATURE-CONDITIONAL MUTANT OF ROUS SARCOMA VIRUS.

Hale AH, Yau TM, Weber MJ
Department of Microbiology, University of Illinois,
Urbana, IL 61801
Biochim Biophys Acta; 443(3):618-622 1976

The increased percentage of oleic acid and decreased percentage of arachidonic acid which occurs in the lipids of chicken embryo fibroblasts transformed by Rous sarcoma virus was shown to be transformation specific rather than a consequence of virus infection. Cells infected with a temperature conditional mutant of Rous sarcoma virus (RSV-T5) had a normal type fatty acid composition when held at the restrictive temperature of 41 degrees C, but had a transformed type fatty acid composition when held at the permissive temperature of 36 degrees C. However, these acyl group changes occurred slowly in the course of transformation, suggesting that they are not a primary event in the genesis of the transformed phenotype. (Author Abstract)

250. ALTERATIONS IN SURFACE GLYCOPROTEINS AND LEVEL OF SIALYLTRANSFERASE OF CELLS TRANSFORMED BY A TEMPERATURE-SENSITIVE MUTANT OF SIMIAN VIRUS 40.

Onodera K, Yamaguchi N, Kuchino T, Aoi Y
The Institute of Medical Science, The University of Tokyo,
P. O. Takanawa, 108, Tokyo, Japan
Proc Natl Acad Sci USA; 73(11):4090-4094 1976

Mouse cells transformed by a temperature-sensitive mutant of simian virus 40 belonging to complementation group A lost their ability to regulate cell growth when grown at the permissive temperature (35 degrees) but showed the low saturation density of cell growth at the restrictive temperature (39.5 degrees) that is characteristic of normal cells in vitro. Biochemical analysis of the membranes of cells grown under the restrictive and the permissive conditions demonstrated no qualitative temperature-dependent differences either in neutral glycolipids or in acidic glycolipids of the cells. Plasma membrane glycoproteins labeled with radioactive glucosamine showed significantly different patterns on both polyacrylamide gel electrophoresis and electrofocusing. When the levels of glycoprotein glycosyltransferases of the cells were examined, the level of sialyltransferase (CMP-N-acetylneuraminic acid: D-galactosyl-glycoprotein N-acetylneuraminyltransferase, EC 2.4.99.1) of the cells grown at the restrictive temperature was

low compared with that of cells grown at the permissive temperature. Our results indicate that the level of sialyltransferase is under the control of the gene A function of simian virus 40 and consequently is related to alterations in the cell surface glycoproteins. (Author Abstract)

B. Transport Properties of Transformed Cells

251. TEMPERATURE-DEPENDENT ALTERATIONS IN SUGAR TRANSPORT IN CELLS INFECTED BY A TEMPERATURE-SENSITIVE MUTANT OF ROUS SARCOMA VIRUS (MEETING ABSTRACT).

Martin GS, Venuta S, Weber M, Rubin H
Dept. Molec. Biol., U. California, Berkeley, CA
Proc Natl Acad Sci USA; 68(11):2739-2741 1971

Chicken-embryo fibroblasts transformed by Rous sarcoma virus (RSV) take up 2-deoxyglucose at a faster rate than uninfected cells, under conditions where transformed and nontransformed cells grow at the same rate. In cells infected by a temperature-sensitive mutant, the stimulation of 2-deoxyglucose uptake is temperature dependent: the increase (3-fold higher) is observed at the permissive (36 C), but not at the nonpermissive (41.5 C) temperature. When infected cells are shifted from the nonpermissive temperature to the permissive temperature, the uptake of 2-deoxyglucose increases from a rate equal to that of uninfected cells to a rate equal to that of cells infected by the wild-type Schmidt-Ruppin Rous sarcoma virus. The reverse change occurs when the infected cells are shifted from the permissive to the nonpermissive temperature. By the use of cytosine arabinoside, an inhibitor of DNA synthesis, it was possible to show that DNA synthesis is neither required for the transformation, which occurs when the infected cells are shifted from the nonpermissive to the permissive temperature, nor for the phenotypic reversion, which occurs in the reverse shift.

252. TEMPERATURE-DEPENDENT ALTERATIONS IN 2-DEOXYGLUCOSE UPTAKE IN RAT CELLS TRANSFORMED BY A COLD-SENSITIVE MURINE SARCOMA VIRUS MUTANT.

May JT, Somers KD, Kit S
Baylor Coll. Med., Houston, TX
Int J Cancer; 11(2):377-384 1973

Cells transformed by Moloney sarcoma virus (MSV) take up 2-deoxyglucose at a faster rate at 39 C than uninfected or Moloney leukemia virus (MoLV)-infected normal rat kidney (NRK) cells. In a sarcoma-virus-transformed cell line, NRK(MSV-1b), whose transformed phenotype is expressed at 39 C (permissive) but not at 33 C (non-permissive), the stimulation of 2-deoxyglucose uptake at 39 C is temperature dependent: the increase is observed when cells are grown at 39 C but not at 33 C. When these cells were shifted from the non-permissive to the permissive temperature, the uptake increased from a rate near that of uninfected cells to a rate half that of NRK cells infected with Moloney sarcoma-leukemia complex (MSV-MoLV). The reverse change occurred when the cells were shifted from the permissive to the non-permissive temperature. Thus in a sarcoma virus-infected rat cell line where the maintenance of the transformed state is dependent on a cold-sensitive viral function, the uptake of 2-deoxyglucose is reduced in the cold-sensitive cells and correlated with the expression of the transformed phenotype.

253. SUGAR TRANSPORT IN CHICK EMBRYO FIBROBLASTS. II. ALTERATIONS IN TRANSPORT FOLLOWING TRANSFORMATION BY A TEMPERATURE-SENSITIVE MUTANT OF THE ROUS SARCOMA VIRUS.

Kletzien RF, Perdue JF
McArdle Lab. Cancer Res., U. Wisconsin Med. Ctr.,
Madison, WI
J Biol Chem; 249(11):3375-3382 1974

Chick embryo fibroblasts infected with the temperature-sensitive mutant of the Rous sarcoma virus, Ts-68, were morphologically transformed when cultured at 37 C, but retained their fusiform morphology when cultured at 41 C. Morphological transformation was accompanied by 2.5- to 4-fold increases in the rate of 2-deoxy-D-glucose uptake. Although the initial rates of 2-deoxy-D-glucose uptake and intracellular phosphorylation in Ts-68-infected fibroblasts were temperature dependent, no differences were found in the initial rates of phosphorylation in homogenates prepared from infected cells cultured at 41 and 37 C. The inhibition of 2-deoxy-D-glucose uptake by cytochalasin B was always paralleled by an equal inhibition of 2-deoxy-D-glucose phosphorylation. Thus, transport was rate-limiting in 2-deoxy-D-glucose uptake at early time points and alterations in transport were responsible for the alterations in sugar uptake in Ts-68-infected chick embryo fibroblasts. The K_m remained constant at 2 and 5 mM at 37 and 41 C for 2-deoxy-glucose and 3-O-methyl-D-glucose, respectively, but the V_{max} was more than 2-fold greater for cells cultured at 37 C. Only the V_{max} for 2-deoxy-D-glucose transport was changed following a 6-hour shift in culture temperature from 41 to 37 C. The similar values calculated for activation energy (17.9 and 17.5 Cal/mole for the 2-deoxy-D-glucose transport reaction in Ts-68-infected cells at 41 and 37 C, respectively) are consistent with the interpretation that the increase V_{max} in cells cultured at 37 C was the result of an increase in the number of transport sites.

254. UPTAKE OF 2-DEOXYGLUCOSE BY BALB/3T3 CELLS: CHANGES AFTER POLYOMA INFECTION.

Eckhart W, Weber M
Salk Inst., San Diego, CA
Virology; 61(1):223-228 1974

The rate of 2-deoxyglucose uptake in exponentially growing and resting BALB/3T3 cell cultures was examined before and after lytic infection with wild-type polyoma virus or two temperature-sensitive polyoma mutants and was compared to rates in cultures of BALB/3T3 cells transformed by simian virus 40 (SV40). The rate of uptake was approximately 3-fold faster in actively growing cells than in resting cells, and 3-fold faster in polyoma or SV40-transformed cells than in actively growing untransformed cells. The uptake of 2-deoxyglucose increased approximately 10-fold after infection of resting cells by polyoma virus and 3-fold after polyoma infection of growing cells. The uptake of 3-O-methylglucose also increased after infection of resting cells, indicating that the changes in the rate of 2-deoxyglucose uptake can be ascribed to changes in the rate of transport of hexose across the cell membrane. The increase in uptake in polyoma-infected cells began about 12 hr after the infection of resting cells at 37 C and occurred in the presence of cytosine arabinoside. The increase occurred after infection at 39 C by polyoma temperature sensitive mutant (ts25), in which viral DNA synthesis is blocked at the restrictive temperature, (39 C). The increase did not occur after infection with ts3, a mutant in which the induction of cellular DNA synthesis is blocked at the restrictive temperature, at 39 C; it did occur after ts3 infection at the permissive temperature (32 C). The results indicate that an early viral gene function is required to

bring about the postulated change in membrane activity during lytic infection.

255. STIMULATION OF 2-DEOXYGLUCOSE UPTAKE IN GROWTH-INHIBITED BALB/C 3T3 AND REVERTANT SV40-TRANSFORMED 3T3 CELLS.

Bradley WL, Culp LA

Sch. Med., Case Western Reserve U., Cleveland, OH
Exp Cell Res; 84(1/2):335-350 1974

Sugar transport in the contact-inhibited BALB/c 3T3 and revertant simian virus 40 (SV40)-transformed 3T3 cells was markedly diminished when these cells became growth-inhibited. Insulin rapidly stimulated transport in both normal 3T3 and revertant cells, when they were contact inhibited, by increasing the Michaelis-Menton kinetic parameter V_{max} , possibly reflecting a rapid increase in the number of transport sites on the surface of these cells. Insulin had no effect on sugar transport in SV40-transformed (SVT2) cells, growing 3T3 cells, or growing revertant cells. Increasing concentrations of fresh fetal calf serum stimulated transport in contact-inhibited 3T3 and revertant cells to levels identical with those in growing cells; this correlated with its ability to 'turn on' DNA synthesis in the vast majority of these cells. This stimulation was maximal at serum concentrations of 35-80%. The mechanisms of stimulation for the two cell types were quite different. Stimulation of transport in 3T3 cells was initially rapid with an increase in V_{max} followed 1 hr later by a second increase resulting from a diminished K_m . Stimulation in the revertant cells proceeded slowly and linearly and was a result of V_{max} with no apparent change in the K_m constant. Although the revertant cells exhibited sugar transport properties which, in general, resembled those in normal 3T3 cells and not those in their parental SVT2 cells, the mechanisms of transport changes were quite different. With regard to the stimulation of transport, fresh fetal calf serum was partially multipotential, while the BALB/c 3T3 cells were not. The serum was modified in that it lost its ability to cause the decrease in K_m ; this correlated with the loss in its ability to induced DNA synthesis in these cells.

256. CELL SURFACE CHANGES AND ROUS SARCOMA VIRUS GENE EXPRESSION IN SYNCHRONIZED CELLS.

Hale AH, Winkelhaak JL, Weber MJ

Dep. Microbiology, Univ. Illinois, Urbana, IL 61801
J Cell Biol; 64(2):398-417 1975

Studies were conducted to determine whether changes associated with growth control and malignant transformation are linked to the cell cycle. Chicken embryo cells, infected with a temperature-sensitive mutant of Rous sarcoma virus, were synchronized by double thymidine block. Cells were examined for cell cycle-dependent alterations in membrane function by determining the rate of transport of 2-deoxyglucose, uridine, thymidine, and mannitol. (3H)2-deoxyglucose was used at 0.5 microCi/ml, uridine and mannitol at 1.0 microCi/ml, and (3H)thymidine at 5.0 microCi/l. Cell cycle-dependent alterations in cell surface morphology were determined by scanning electron microscopy. Cells were also examined for the ability of tumor virus gene expression to induce a transformation-specific change in membrane function. Infected cells were shifted from the restrictive to the permissive temperature and monitored for a transformation-specific increase in 2-deoxyglucose transport rate. It was determined that the high rate of 2-deoxyglucose transport seen in transformed cells and the low rates of 2-deoxyglucose and uridine transport characteristic of density-inhibited cells do not occur in normally growing cells as they traverse the cell cycle. Although there are cell cycle-dependent changes in surface morphology, they are not reflected in corresponding changes in membrane function. Tumor virus gene expression

can alter cell membrane function at any stage in the cell cycle and without progression through the cell cycle. It is concluded that cell surface changes associated with growth control and malignant transformation are not cell cycle dependent.

257. TRANSPORT CHANGES ASSOCIATED WITH GROWTH CONTROL AND MALIGNANT TRANSFORMATION.

Weber MJ, Hale AH, Yau TM, Buckman T, Johnson M, Brady TM, La Rossa DD

Dept. Microbiology, Univ. Illinois, Urbana, IL 61801
J Cell Physiol; 83(4):711-722 1976

The rate of uptake of nutrients and ions in chick embryo fibroblasts that were either density-inhibited, growing exponentially, or transformed by Rous sarcoma virus was determined. When normal growing cells were compared to transformed cells, only one change was detected: uptake of 2-deoxyglucose and 3-O-methylglucose was increased three- to five-fold in the transformed cells. ³H-ouabain binding showed that there were fewer ouabain binding sites on the density-inhibited cells than on the growing cells. The uptake of ³H-2-deoxyglucose was measured at temperatures from 43 to 0 C, and the data were utilized to construct Arrhenius plots. The slope of the plot was steeper for the transformed cultures than for the normal cultures. To examine the time course of appearance of the tumor virus-induced fatty acid alterations, temperature-conditional mutant of Rous sarcoma virus (RSV-T5) was used. Chick cells infected with RSV-T5 and held at 36 C had a transformed phenotype and had a fatty acid composition typical of transformed cells, but the culture held at 41 C had a normal phenotype and a normal-type fatty acid composition. At time zero, cultures were shifted to the opposite temperature. At 10 and 26 hr after the shift, cultures were taken for analyses. Although the switch in the capacity to take up 2-deoxyglucose was complete within 26 hr, the changes in fatty acid composition were less than half complete. The addition of proteases (including plasmin) to normal, density-inhibited cultures did not stimulate them to take up 2-deoxyglucose at a rate much above that characteristic of normal, growing cells. Addition of dibutyl cyclic AMP to cells transformed by RSV-T5 restored the transformed level of 2-deoxyglucose uptake to normal. The results indicate that there are two classes of transport control in cultured chick cells. (50 Refs)

258. REGULATION OF SUGAR TRANSPORT IN CHICK EMBRYO FIBROBLASTS AND IN FIBROBLASTS TRANSFORMED BY A TEMPERATURE-SENSITIVE MUTANT OF THE ROUS SARCOMA VIRUS.

Kletzien RF

Sidney Farber Cancer Center, 44 Binney St., Boston, MA 02115

J Cell Physiol; 89(4):723-728 1976

The regulation of sugar transport in transformed (by a temperature-sensitive mutant of Rous sarcoma virus) and normal chick embryo fibroblasts (CEF) was assessed. The addition of fetal calf serum to serum-deficient CEF resulted in a biphasic increase in the sugar transport rate. The first increase in transport occurred within 10 min of serum addition, and it was not blocked by prior addition of actinomycin D or cycloheximide. The transport started to increase again 45-60 min after serum addition. The second increase was blocked by cycloheximide but not by actinomycin D. Cordycepin (10 ug/ml) blocked the second phase of the increase in sugar transport as effectively as did cycloheximide. The effects of actinomycin D, cordycepin, and cycloheximide on the transport increase induced by hexose starvation were determined. In contrast to the increases induced by serum stimulation of CEF, actinomycin as well as cordycepin and cycloheximide were effective in blocking this transport increase. The data indicate

the rapidity with which transport activity changed when Ts68-infected CEF were shifted from the nonpermissive (41 C) to the permissive (37 C) temperature for transformation or vice versa. The greatest rate of change occurred in the first 6 hr after a temperature shift. Actinomycin D did not block the increase in transport when cultures were shifted from 41 to 37 C, but cycloheximide did. Ts68-infected CEF cultured at 41 C responded to serum removal and addition, as did uninfected CEF. However, Ts68-infected CEF cultured at 37 C did not exhibit changes in the max velocity for transport in response to serum addition or removal. These cultures continuously expressed an elevated transport rate. When Ts68-infected CEF were incubated in glucose-free medium at 37 or 41 C, the max velocity differences for sugar transport disappeared between the transformed and untransformed cells. The key to understanding transformation may lie in understanding the means by which the temperature-sensitive viral gene product alters regulation. (17 Refs)

259. UPTAKE OF EXOGENOUS METABOLITES BY VIRUS-TRANSFORMED CELLS: CHANGES INDUCED BY TEMPERATURE AND PH.

Bader JP, Lew MA, Brown NR
Chemistry Branch, NCI, NIH, Bethesda, MD 20014
Arch Biochem Biophys: 175(1):1.6-208 1976

Rates of uptake of a variety of exogenous metabolites were measured in chick embryo (CE) cells and in CE cells transformed by Rous sarcoma virus, Bryan high-titer strain (RSV-BH). Deoxyglucose uptake was greater in transformed than in nontransformed cells. Less substantial increases in uptake of galactose, uridine, thymidine, and leucine were found in transformed cells. A mutant of RSV-BH, RSV-BH-Ta, allowed infected cells to behave as nontransformed cells at 41 C, but when shifted to 37 C, cells became transformed. In RSV-BH-Ta cells shifted from 41 to 37 C increased capacities for uptake of all tested compounds were prevented by actinomycin D or cycloheximide, but not cytosine arabinoside, demonstrating that new RNA and protein synthesis was required for uptake enhancement. The pH of the culture medium over the pH range 6.6 to 7.8 was critical to cellular uptake capacity for deoxyglucose, but had little influence on capacity for uptake of galactose, uridine, thymidine, or leucine. Changes induced by pH occurred in both nontransformed and transformed cells, and these changes occurred in the presence of actinomycin D or cycloheximide. Both the V and Km of deoxyglucose uptake changed when cells became transformed by RSV-BH or RSV-BH-Ta. However, only V changed with changes in pH. Neither the lower Km characteristic of transformed cells nor the higher Km of nontransformed cells were affected by pH. (45 refs)

C. Cytoskeletal Structures in Transformed Cells

260. CONTACT-INHIBITED REVERTANT CELL LINES ISOLATED FROM SV40-TRANSFORMED CELLS. IV. MICROFILAMENT DISTRIBUTION AND CELL SHAPE IN UNTRANSFORMED, TRANSFORMED, AND REVERTANT BALB/C 3T3 CELLS.

McNutt NS, Culp LA, Black PH
Massachusetts Gen. Hosp., Boston, MA
Cell Biol: 56(2):412-428 1973

A comparison was made of the ultrastructure of the cell periphery in three cloned cell lines: untransformed BALB/c 3T3 cells, SV40-transformed BALB/c 3T3 cells, and revertant cells obtained from the transformed cell line by a selection technique utilizing concanavalin A. Both thin-section and surface replication techniques were used for in situ examination of the cell lines. Microfilaments, 70 angstroms in diameter (called alpha filaments), were abundant in untransformed and revertant cell lines, particularly in the

anterior expansions of the cells, which tended to have many microvilli and small pseudopodia. Alpha filaments were diminished in the anterior expansions of transformed cells, which contained large blunt pseudopodia and relatively few microvilli. Surface replicas confirmed the impression gained from thin sections that transformed cells have a greater proportion of their cell surface involved in bulging pseudopodia than either untransformed or revertant cells. Since alpha filaments bind heavy meromyosin and are similar to F-actin, these filaments are thought to be important in cell motility. These observations suggest that a close relationship exists between decreased alpha filaments, bulging pseudopodia, and loss of contact inhibition of movement in transformed cells.

261. PATTERNS OF ORGANIZATION OF ACTIN AND MYOSIN IN NORMAL AND TRANSFORMED CULTURED CELLS (MEETING ABSTRACT).

Pollack R, Osborn M, Weber K
Cold Spring Harbor Lab., NY 11724
Proc Natl Acad Sci USA: 72(3):994-998 1975

Actin and myosin in normal and transformed cell cultures were examined by immunofluorescence using antibody directed against actin and antibody directed against myosin. Immunofluorescence revealed the position of actin and myosin within a series of normal, simian virus 40 (SV40)-transformed and revertant cells of rat and mouse origin. Cell lines included: normal mouse 3T3, SV101 (a 3T3 line transformed by SV40), F1SV101 (a density-sensitive revertant of SV101), A2 and LS (serum-sensitive revertants of SV101), primary rat embryo (RE), SV40-transformed RE (including SVRE 9, SVRE 12, and WT4), and tsA SV40-transformed RE (tsA28-3). The relationship between transformation and the loss of actin-containing sheaths was correlated with anchorage dependence, cell density and growth in low serum concentration. Loss of actin-containing sheaths and anchorage occurred in the following lines: SV101, A2, LS, SVRE9, WT4, and TSA28-3. Data obtained with actin antibody showed that different cells display different distributions of actin-containing sheaths. SV40 transformation of 3T3 cells is accompanied by decreased actin-containing sheaths, and the reversion of SV101 to a flat revertant line (F1 SV101) shows increased actin-containing sheaths. The results of myosin antibody on various cell lines were qualitatively similar to those obtained with actin antibody. Of the three parameters studied, only anchorage-dependence is correlated with the loss of actin-containing sheaths.

262. LOCALIZATION AND DISTRIBUTION OF ACTIN FIBERS IN NORMAL, TRANSFORMED AND REVERTANT CELLS. (PP. 363-369)

Weber K, Lazarides E, Goldman RD, Vogel A, Pollack R
Tumor Viruses Cold Spring Harbor Symposia on
Quantitative Biology. Cold Spring Harbor Lab. Cold
Spring Harbor, NY, vol. 39, 1975

An antibody against electrophoretically purified SDS-denatured actin from mouse 3T3 cells (SV40-transformed clone SV101) was used to examine normal rat embryo cells, the growth-controlled mouse cell lines 3T3 and BALB 3T3, a variety of virus-transformed clones of these lines, and revertants derived from the transformants for the presence and distribution of actin. Evidence suggests that the major SV101 protein species with an electrophoretic molecular weight of 45,000 is indeed actin. The antibody cross-reacts with actin from cells from a variety of different species. Both light microscopy and darkfield UV optics revealed a variety of fibers in most 3T3 cells; these fibers apparently contain actin. In the absence of any restriction on growth, normal, untransformed fibroblasts were found to contain "actin cables." Transformed clones isolated after infection with SV40 or MSV showed a drastic loss of strong actin fibers. The two

density-sensitive revertants F1SV and M22 showed a remarkable recovery of the cabling pattern originally present in 3T3 or Balb but lost upon viral transformation. However, the serum-sensitive revertant LS showed a pattern similar to that of SV101 and was therefore distinctly different from the other two revertants. Neither serum concentration nor, by implication, the level of cellular cyclic AMP seemed to have any marked effect on the expression of actin cables. These results suggest at least four different levels of cellular actin organization: thick actin bundles, thinner bundles or threads, shorter and thinner actin aggregates, and G actin or soluble actin.

263. TEMPERATURE-SENSITIVE CHANGES IN SURFACE MODULATING ASSEMBLIES OF FIBROBLASTS TRANSFORMED BY MUTANTS OF ROUS SARCOMA VIRUS.

Edelman GM, Yahara I
Rockefeller Univ., New York, NY 10021
Proc Natl Acad Sci USA; 73(6):2047-2051 1976

The hypothesis that surface modulating assemblies (SMA) containing microfilaments and microtubules are altered after cellular transformation was tested on cells infected with temperature-sensitive (ts) mutants of avian sarcoma virus. Untransformed cells (mouse 3T3 and chick fibroblasts), cells transformed by simian virus 40 (SV 3T3), and chick fibroblasts infected with Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV-A-infected cells) were first compared for differences in microfilament and microtubule patterns after treatment with fluorescein-labeled antibodies to actin and tubulin. Transformed cells showed disappearance of ordered stress microfilaments and thickened or diffuse alterations of microtubular arrays. At restrictive temperatures (41°C), chick fibroblasts infected with ts 68 of Rous sarcoma virus showed normal patterns of stress filaments and radial microtubular arrays originating in one or two centrioles. At permissive temperatures (37°C), these patterns were disordered and resembled those of SR-RSV-A-infected cells. After a shift from 41°C to 37°C, the changes in microtubules were observed in the majority of cells within 1 hr. These changes were reversible and did not result from the inability of tubulin to polymerize. In ts 68-infected cells at permissive temperatures, concanavalin A induced much less surface modulation (inhibition of receptor mobility) than at restrictive temperatures. These results suggest that cellular transformation alters both the structure and function of SMA and prompt the hypothesis that products of viral transforming genes may affect SMA with a consequent loss of growth control.

264. CHANGES IN MICROFILAMENT ORGANIZATION AND SURFACE TOPOGRAPHY UPON TRANSFORMATION OF CHICK EMBRYO FIBROBLASTS WITH ROUS SARCOMA VIRUS.

Wang E, Goldberg AR
The Rockefeller University, New York, NY 10021
Proc Natl Acad Sci USA; 73(11):4065-4069 1976

A series of morphological changes occurred when chick embryo fibroblasts infected with the NY68 mutant of Rous sarcoma virus were shifted from nonpermissive temperature (41 degrees) to permissive temperature (37 degrees). We observed three distinct stages in cell morphology and surface topography that were correlated with a reduction in the organization and assembly of actin-containing microfilament bundles. Our observations suggest that control of microfilament organization and surface topography are responsive to the presence of a functioning transforming gene (src) product of Rous sarcoma virus. (Author Abstract)

265. REVERSION FROM TRANSFORMED TO NORMAL PHENOTYPE BY INHIBITION OF PROTEIN SYNTHESIS IN RAT KIDNEY CELLS INFECTED WITH A TEMPERATURE-SENSITIVE MUTANT OF ROUS SARCOMA VIRUS.

Ash JF, Vogt PK, Singer SJ
Department of Biology, University of California at San Diego, La Jolla, CA 92093
Proc Natl Acad Sci USA; 73(10):3603-3607 1976

By the use of a rat kidney cell line infected with a temperature-sensitive Rous sarcoma virus, we have shown that, at permissive temperatures where the cells are transformed, concanavalin A induces a clustering of its cell membrane receptors into patches, and the intracellular smooth muscle myosin-like protein is in a disordered state. By contrast, with infected cells grown at nonpermissive temperatures, the addition of concanavalin A does not alter the uniform distribution of its receptors, and the smooth muscle myosin-like protein is arranged in an ordered filamentous structure. These results are consistent with the hypothesis that the myosin protein is part of an intracellular aggregating-disaggregating complex. In the normal cell it is in its aggregated state and inhibits the lateral mobility of the concanavalin A receptors in the membrane; in the transformed cell the complex is relatively disaggregated and permits the concanavalin A receptors to be mobile. The addition of protein synthesis inhibitors to infected cells grown at the permissive temperature causes the cells to change from the transformed phenotype to the normal. Removal of the reversible inhibitors causes the cells to revert to the transformed phenotype. These results show that (i) protein synthesis, presumably of an unstable product of the transforming gene of the temperature-sensitive virus, is required to maintain the transformed state in these infected cells at the permissive temperature; and (ii) protein synthesis is not required for the intracellular myosin-containing complex to revert from its disordered transformed state to its ordered normal state. This suggests that the product of the transforming gene directly or indirectly causes the disaggregation of the myosin-containing complex in the process of transformation. (Author Abstract)

266. MICROFILAMENT BUNDLES AND CELL SHAPE ARE RELATED TO ADHESIVENESS TO SUBSTRATUM AND ARE DISSOCIABLE FROM GROWTH CONTROL IN CULTURED FIBROBLASTS.

Willingham MC, Yamada KM, Yamada SS, Pouyssegur J, Pastan I
National Cancer Institute, Bethesda, MD 20014
Cell (Camb); 10(3):375-380 1977

The distribution of microfilament bundles in cells was examined using antibodies to fibroblast myosin and indirect immunofluorescence microscopy. There is no correlation between the presence of bundles of microfilaments and normal growth control. A normal cell line (BALB/c 3T3) cultured on a poorly adhesive substratum showed no microfilament bundles. Similarly, a mutant cell line (AD6) with normal growth, but a rounded shape due to defective adhesiveness to substratum, showed no bundle formation. On the other hand, two transformed cell lines with a flat morphology (Swiss SV3T3 and Balb MSV-85) showed extensive bundle formation. When a transformed cell line with poor adhesiveness (MC5-5) was treated with CSP (a major surface glycoprotein of normal cells) which increases adhesiveness to substratum, the cells formed extensive microfilament bundles without any decrease in growth. We conclude that the distribution of microfilament bundles is related to adhesiveness to substratum and cell shape but not to growth properties. (18 Refs) (Author Abstract)

267. EXPRESSION OF VIRUS SPECIFIC MORPHOLOGICAL CELL TRANSFORMATION INDUCED IN ENUCLEATED CELLS.

Beug H, Peters JH, Graf T

Max-Planck-Institut für Virusforschung, Biologisch-Medizinische Abteilung, Spemannstr. 35/III, D-7400 Tübingen, W. Germany

Z Naturforsch A; 31(11/12):766-768 1976

The role of nuclear components in the functioning of the transformation-inducing viral gene product, as judged by changes in cell morphology, was investigated using chick embryo fibroblasts. The fibroblasts, infected by a temperature-sensitive mutant of Rous sarcoma virus, were enucleated with Cytochalasin B. The cytoplasts were still able to transform morphologically when shifted from nonpermissive to permissive temperatures and to revert to their normal morphology when shifted from permissive to nonpermissive temperatures. These results suggest that the primary target for the transforming gene product does not reside in the nucleus but in the cytoplasm or in the membrane. Since the morphology of a cell appears to be largely determined by the organization of its cytoskeleton, this could be the possible target. It remains to be shown, however, that alterations of the microfilament-microtubuli system take place in enucleated fibroblasts shifted from one temperature to another. (5 Refs)

D. Cyclic Nucleotides in Transformed Cells

268. DIFFERENCE IN THE CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE LEVELS IN NORMAL AND TRANSFORMED CELLS.

Sheppard JR

U. Colorado Med. Sch., Denver, CO
Nature New Biol; 236(61):14-16 1972

Steady-state cyclic 3',5'-adenosine monophosphate (cAMP) levels in normal and transformed cells were determined by an in vitro assay, and the effect of several biochemicals on these levels was studied. These cAMP levels were at least two times higher in normal density-dependent (3T3) cells as compared with virus-transformed (py 3T3) and spontaneously transformed (3T6) cells. Pollack's flat revertants (fLSV and fpy), which exhibit normal growth, had levels of cAMP in the range of normal 3T3 cells. When cAMP levels in 3T3, 3T6 and py3T3 cells were studied over a seven-day growth period, little change was seen as the cells reached confluency, indicating that cAMP levels in these cells were not affected by their state of growth. When insulin, 50% calf serum or 0.05% trypsin, which have been shown to stimulate confluent normal cells to further growth, were added to confluent 3T3, 3T6 or py3T3 cultures, cAMP levels were depressed two- to threefold within five min. Prostaglandin E₁, which may inhibit cellular growth in virally and spontaneously transformed cells, increased cAMP levels within five min; the extent of stimulation was greater (sixfold) in the 3T3 cells, as compared with the transformed 3T6 and py3T3 cells (twofold). cAMP levels had returned to basal values by three hr. Prostaglandin F₂α had no effect on cAMP levels in 3T3, 3T6 or py3T3 cells. These results are consistent with the hypothesis that cAMP plays a role in the control of cellular growth.

269. A MUTATION IN A ROUS SARCOMA VIRUS GENE THAT CONTROLS ADENOSINE 3',5'-MONOPHOSPHATE LEVELS AND TRANSFORMATION

Otten J, Bader J, Johnson GS, Pastan I

Natl. Cancer Inst., Bethesda, MD
J Biol Chem; 247(5):1632-1633 1972

Cultured chick embryo cells were infected with a temperature-sensitive mutant of the Bryan strain of Rous sarcoma virus (RSV-Ta). RSV-Ta is capable of infecting cells and replicating at both 40.5 C and 36 C; however, at 40.5 C it is unable to transform the cell. When dibutyryl-cAMP (Bt2-cAMP) (1.2 mM) and theophylline (1 mM) were added to infected chick cell cultures 40.5 C two hr before the temperature was lowered to 36 C, the cells retained their normal morphology for at least the following 12 or 24 hr. Phosphodiesterase inhibitors partially blocked the transformation at 36 C. Papaverine (0.2 mM) or 1-methyl-3-isobutylxanthine (1 mM) each in combination with Bt2-cAMP (1.2 mM) completely blocked transformation. Adenosine monophosphate showed no blocking effect. In infected cells at 40.5 C, the level of cAMP was in the range of normal cells (1.7 plus or minus 15 pmoles per microg nucleic acid) but when cells were shifted to 36 C for 12 hr, cAMP levels fell dramatically (20 pmoles per microg of nucleic acid). Levels of cAMP were low in cells transformed by wild-type RSV at both temperatures (34 to 39 plus or minus 3 pmoles per microg of nucleic acid). Levels of cAMP in normal fibroblasts were unaffected by temperature (210 pmoles per microg of nucleic acid). Within 20 min after changing infected cells from a temperature of 40.5 C to 36 C, the level of cAMP fell by about 50%. Levels remained in this range for approximately six hr. After 12 hr cAMP levels again began to decrease. These results suggest that an early event in transformation leads to a decreased level of cAMP, and the decreased cAMP level results in some of the unique properties of transformed cells.

270. TRANSFORMATION OF CHICK-EMBRYO FIBROBLASTS BY WILD-TYPE AND TEMPERATURE-SENSITIVE ROUS SARCOMA VIRUS ALTERS ADENYLATE CYCLASE ACTIVITY (MEETING ABSTRACT).

Anderson WB, Johnson GS, Pastan I

Natl. Cancer Inst., Bethesda, MD
Proc Natl Acad Sci USA; 70(4):1055-1059 1973

The activity of the enzyme adenylate cyclase, a component of the plasma membrane, has been determined in chick-embryo fibroblasts and in cells transformed by either Bryan high-titer strain of Rous sarcoma virus (RSV-BH) or a temperature-sensitive mutant of this virus (RSV-BH-Ta). Adenylate cyclase activity is reduced in cells transformed by the wild-type virus and also by the temperature-sensitive mutant when the cells are grown at the permissive temperature (37 C). Transformation results in an altered affinity (K_m) for the substrate (MgATP). The apparent K_mATP is 0.23 mM in normal cells and 1.1 mM in cells transformed with wild-type virus. The K_mATP of the cells infected with RSV-BH-Ta is 0.67-1.0 mM at 37 C and 0.28 mM at 42 C. The enzyme from normal cells appears to have two binding sites for Mg⁺⁺, one at the catalytic site and a second at a regulatory site. Transformation by RSV-BH or RSV-BH-Ta (37 C) apparently alters this second Mg⁺⁺ site. A decrease in adenylate cyclase activity occurs within 10 min after cells infected with RSV-BH-Ta are shifted from 42 C to 37 C; the activity falls to one-half that of normal cells 30 min after the temperature shift. These observations indicate that a viral function lowers cyclic AMP content by lowering the activity of adenylate cyclase, probably through some modification of the plasma membrane.

271. ADENYLATE CYCLASE ACTIVITY IS DECREASED IN CHICK EMBRYO FIBROBLASTS TRANSFORMED BY WILD TYPE AND TEMPERATURE SENSITIVE SCHMIDT-RUPPIN ROUS SARCOMA VIRUS.

Anderson WB, Lovelace E, Pastan I
Natl. Cancer Inst., Bethesda, MD
Biochem Biophys Res Commun; 52(4):1293-1299 1973

Adenylate cyclase levels were studied in normal and Schmidt-Ruppin strain Rous sarcoma virus (SR-RSV)-infected chick embryo fibroblasts by a radioassay. Cells infected with wild-type virus showed 40-50% less adenylate cyclase activity than normal cells. Change in growth temperature had no effect on enzyme levels. Cells infected with a temperature-sensitive mutant had decreased adenylate cyclase levels at the permissive temperature and normal levels at the nonpermissive temperature. Enzyme activity decreased slowly over a 24 hr period when nonpermissive cells were switched to the permissive temperature. The decreased enzyme activity was not due to an altered (K_m (ATP) nor to a change in the kinetics of Mg^{++} activation, and was not observed when the enzyme was assayed in the presence of NaF. Several of these properties of SR-RSV infected cells differed from those of cells infected by the Bryan high-titer strain RSV, and it was thus concluded that transformation by SR-RSV reduces enzyme activity by a different mechanism than that of the Bryan strain.

272. CYCLIC AMP AND CYCLIC GMP CONCENTRATIONS IN SERUM- AND DENSITY-RESTRICTED FIBROBLAST CULTURES (MEETING ABSTRACT).

Moens W, Vokaer A, Kram R
Universite Libre de Bruxelles, Departement de Biologie
Moleculaire, 67, rue des Chevaux, 1640 Rhode St.
Genese, Belgium
Proc Natl Acad Sci USA; 72(3):1063-1067 1975

The concentrations of 3',5'-cyclic AMP (cAMP) and 3',5'-cyclic GMP (cGMP) in serum- and density-inhibited cultures of 3T3 cells, SV40-transformed 3T3 cells (SV3T3-101), and four revertant lines derived from SV101. Revertant clones F1SV101 and BuSV2 were density dependent, and the serum-dependent clones LSV2 and A SV5 were unable to grow at low serum concentrations (1%) and in gamma-depleted 10% calf serum, respectively. The intracellular cAMP and cGMP levels were assayed in cultures growing in medium containing 10% or 0.5% calf serum and in cultures grown to confluence in 10% serum. The cAMP levels in the growing SV101 cultures were about 1/2 that observed in the 3T3 cultures and the cGMP levels in the transformed cultures were about twice those in the untransformed cells. The cAMP pattern of A SV5 was similar to that of SV101, while the cAMP patterns in the other revertants were similar to that of 3T3. The cGMP concentrations in the serum-dependent revertants were similar to that of 3T3, while those in the density-dependent revertants were similar to that of SV101. Density-dependent inhibition of growth was correlated with reduced cGMP levels in 3T3 and the four revertants, while the levels in SV101 were only slightly decreased. The cAMP levels decreased slightly in all cultures at confluence. In medium containing 0.5% serum, the serum-dependent lines showed a greater increase in intracellular cAMP than did the serum-insensitive lines, while the cGMP levels were greatly reduced in all except SV3T3 by SV101. Serum readdition to all serum-dependent lines was followed by a rapid decrease in cAMP and an increase in cGMP. The magnitude of these responses was decreased in SV101 and the density revertants. The data suggest that decreased cGMP concentrations might specifically mediate sensitivity to density-dependent inhibition of growth and that serum- and density-dependence are distinct.

273. STRAIN SPECIFICITY OF CHANGES IN ADENYLATE CYCLASE ACTIVITY IN CELLS TRANSFORMED BY AVIAN SARCOMA VIRUSES.

Yoshida M, Owada M, Toyoshima K
Fac. Pharm. Sci., Univ. Tokyo, Japan
Virology; 63(1):68-76 1975

The relationship between viral functions and changes in enzyme activities was examined by measuring the adenylate cyclase and cyclic AMP phosphodiesterase activities in chick embryo fibroblasts transformed by the avian sarcoma virus B77, the Prague and Schmidt-Ruppin strains of Rous sarcoma virus, and their temperature-sensitive mutants. Infected chick embryo fibroblasts were transferred once before tests to induce extensive transformation of cultured cells. Enzyme activity was determined by paper chromatography of cell homogenates. With both normal chick embryo fibroblasts and transformed cells, adenylate cyclase activity was linear for at least ten minutes, and phosphodiesterase was linear for more than 30 min at 37 C. The transformed chick embryo fibroblasts had lower activities of adenylate cyclase, but they had the same activity of cyclic AMP phosphodiesterase as control cells. The cells infected with their temperature-sensitive mutants in the transforming capacity also had reduced cyclase activity when grown at the permissive temperature (36 C) but normal activity when grown at the nonpermissive temperature (41 C), indicating a close correlation between viral transformation and cellular adenylate cyclase activity. The dependencies of adenylate cyclase activity on ATP concentration in normal and transformed cells with 10 mM $MgCl_2$ were compared, and the extent of stimulation of the activity by NaF was measured. The K_m value for ATP, Mg^{++} dependency, and sensitivity to NaF of the adenylate cyclase activity reduced by viral transformation varied with the viral strain. The enzyme of B77-transformed cells at 41 C had a higher K_m for ATP (0.29 mM) than that of normal cells (0.14 mM). Transformation by the Prague or Schmidt-Ruppin strains of Rous sarcoma virus had no effect on the K_m value of adenylate cyclase for ATP. None of the virus strains induced significant change in the Mg^{++} concentration dependence of the cellular cyclase activity. NaF stimulated the adenylate cyclase activity of normal cells 4 to 8-fold. NaF (10 mM) stimulated the cyclase activity of cells transformed by B77 or the Prague strain of Rous sarcoma virus as much as that of normal fibroblasts, but caused more stimulation in cells transformed with the Schmidt-Ruppin strain of Rous sarcoma virus than in normal fibroblasts. It is concluded that the changes in enzyme activity on transformation are specific to the virus strain and are not related to the virus subgroup.

274. CYCLIC AMP-MEDIATED TRANSFORMATION OF RAT CELLS TRANSFORMED BY TEMPERATURE-SENSITIVE MOUSE SARCOMA VIRUS.

Somers KD, Rachmeler M, Christensen M
Eastern Virginia Medical Sch., Norfolk, VA 23507
Nature (Lond); 257(5521):58-59 1975

To examine the relationship between the level of adenosine 3',5'-cyclic AMP (AMP) and the expression of transformed phenotype by virus-transformed cells, three cell lines were studied: normal rat kidney cells (NRK); NRK cells transformed by Moloney sarcoma-leukemia virus NRK(MSV-MLV); and NRK cells transformed by a cold-sensitive mutant of MSV, NRK(MSV^{ts}). After a ten-minute exposure at 35 C to 0.01 mM 8-bromo-cyclic AMP (8-Br-cyclic AMP), morphological change was noted in NRK(MSV-1b) cells, that reached maximum expression by one hour. The effect was to change the phenotype from that present at the nonpermissive temperature of 33 C (cells flattened and poorly refractile) to that characteristic of cells grown at 39 C, the permissive

temperature (cells rounded, highly refractile). When using 0.4 mM N₆O₂'-dibutyl cyclic AMP (db-cyclic AMP) plus theophylline at 33°C, changes were apparent after three hours and maximized by 24 hr. In both cases, 100% of the cells changed, and remained altered as long as the additive was present, up to 72 hr. Removal of the cyclic AMP analogues resulted in a rapid reversal (within one hour) from the transformed to the normal phenotype. Changes were independent of growth phase. The addition of cyclic AMP analogues to uninfected NRK cells and transformed NRK(MSV-MLV) cells provoked no change, regardless of temperature (33, 36, or 39°C). Cyclic GMP and other cyclic nucleotides produced no change in NRK(MSV-1b) cells. Levels of cyclic AMP measured by radioimmunoassay were three times as high at permissive as at nonpermissive temperatures. Temperature shifts produced marked changes: levels increased approximately three-fold within five hours after a shift from nonpermissive to permissive temperatures; in shift-down experiments, levels fell 50% within 24 hr. Under similar conditions, no significant differences for NRK or transformed NRK(MSV-MLV) cells were found. It is concluded that this system is an important exception to mechanisms proposed for the role of cyclic AMP in the expression of the transformed state, which postulate decreased cyclic AMP levels for transformed cells.

275. CHANGES IN ADENYLATE CYCLASE ACTIVITY AND MEMBRANE POLYPEPTIDES OF CELLS TRANSFORMED WITH AVIAN SARCOMA VIRUSES. (PP. 205-215)

Yoshida M, Isaka T, Ikawa Y, Owada M, Toyoshima K. Control Mechanisms in Cancer, Progress in Cancer Research and Therapy. Criss WE, Ono T, Sabine JR, ed. New York, Raven Press, Vol 1, 1976

Studies of the effects of avian sarcoma viruses (ASV) on adenylate cyclase activity in chick embryo fibroblasts (CEF) and duck embryo fibroblasts (DEF) are reviewed. All the ASV tested, including B77 and various strains of Rous sarcoma virus, reduced adenylate cyclase activity in transformed CEF; analysis of temperature-sensitive virus mutants showed that the reduction was related to virus-induced transformation. Characteristics of enzyme activity, such as K_m , ATP level, and Mg²⁺ and Na⁺ dependence, in the transformed cells showed virus-strain specificity. This virus specificity was confirmed in DEF transformed with the same group of viruses. Changes in adenylate cyclase activity were not correlated with cyclic AMP levels, which were unaffected in transformed CEF but reduced in transformed DEF. The virus-strain specificity observed in the two cell types indicates that changes in the properties of the adenylate cyclase system do not result from induction and functioning of endogenous virus genes, but reflect the viral function used for cell transformation. (21 Refs)

276. CALCIUM-DEPENDENT REGULATORY PROTEIN OF CYCLIC NUCLEOTIDE METABOLISM IN NORMAL AND TRANSFORMED CHICKEN EMBRYO FIBROBLASTS.

Watterson DM, Van Eldik LJ, Smith RF, Vannaman TC. Dept. Microbiology and Immunology, Duke Univ. Medical Center, Durham, NC 27710. Proc Natl Acad Sci USA: 73(8):2711-2715 1976

The concentration of a calcium-binding protein modulator of 3',5'-cyclic-nucleotide phosphodiesterase activity was increased in chicken embryo fibroblasts upon transformation by Rous sarcoma virus. This modulator protein from fibroblasts, which has roughly the same molecular size, charge, and functional properties as that isolated from chicken brain, comprised approximately 1.32% of the soluble protein in homogenates of fibroblasts infected and transformed by Rous sarcoma virus. In comparison, the modulator comprised approximately 0.30% of the soluble

protein in homogenates of normal fibroblasts from confluent cultures and 0.36% of the soluble protein in homogenates of fibroblasts infected with a transformation-defective mutant of Rous sarcoma virus. Modulator levels in normal fibroblasts at subconfluent cell densities were 0.42%-0.76% of the homogenate soluble protein; i.e., between that found in confluent normal fibroblasts and in fibroblasts transformed by Rous sarcoma virus. These observations suggest that the modulator protein levels are elevated under conditions in which chicken embryo fibroblasts are undergoing rapid growth and have decreased cyclic AMP levels.

E. Growth Properties/Cell Cycle Characteristics of Transformed Cells

277. BEHAVIOR OF TISSUE CULTURE CELLS INFECTED WITH POLYOMA VIRUS (MEETING ABSTRACT).

Dulbecco R. Salk Inst. Biol. Stud., San Diego, CA. Proc Natl Acad Sci USA: 67(3):1214-1220 1970

Induction of movement, cell survival, mitotic rates, growth parameters, and cell morphology of tissue culture cells (3T3 and BALB/c-3T3 lines) infected with the oncogenic polyoma virus or its temperature-sensitive mutant (Ts-a) were studied. In medium containing 0.15% horse serum the induction of movement across a wound (produced with a glass rod) occurred simultaneously with the induction of DNA synthesis. Infection with Ts-a protected the cells from death at suboptimal serum concentrations, but there was no difference in cell survival between polyoma virus- or mock-infected cultures. Mitosis decreased in mock-infected cells after 20 hr but persisted in Ts-a-infected cultures. The wound serum requirement and topoinhibition of the mock-infected cells were decreased in polyoma virus-infected and Ts-a-infected cells (60-90%). The flat, round-shaped cells of serum-starved 3T3 or BALB/c-3T3 cultures became thick and elongated after infection, similar to changes that occur in cell transformation.

278. TRANSFORMATION BY POLYOMA VIRUS ALTERS EXPRESSION OF A CELL MUTATION AFFECTING CYCLE TRAVERSE.

Burstin SJ, Basilico C. Department of Pathology, New York University School of Medicine, New York, NY 10016. Proc Natl Acad Sci USA: 72(7):2540-2544 1975

A temperature-sensitive mutant of hamster BHK 21 [3] cells, tsAF8, which at 39 degrees becomes arrested in the G1 (G0) phase of the cell cycle, is phenotypically altered with respect to temperature sensitivity after transformation with polyoma virus. Polyoma transformation does not produce reversion to a non-temperature-sensitive phenotype but causes increased entry into S and increased rate of cell death at the nonpermissive temperature, compared to untransformed tsAF8 cells. The increased frequency of cells synthesizing DNA is not accompanied by an increased frequency of mitosis, since most of the polyoma-transformed tsAF8 cells that synthesize DNA at the nonpermissive temperature do not divide. At the permissive temperature, polyoma-transformed tsAF8 cells, unlike tsAF8, also lose viability when exposed to other methods of arresting cells in G1. The most likely explanation for this phenomenon is that polyoma virus transformation interferes with the cellular response to this mutation as well as to other conditions that cause cell cycle arrest in G1. (Author Abstract)

279. RESTING STATE IN NORMAL AND SIMIAN VIRUS 40 TRANSFORMED CHINESE HAMSTER LUNG CELLS.

Martin RG, Stein S

Lab. Molecular Biology, Natl. Inst. Arthritis, Metabolism and Digestive Diseases, NIH, Bethesda, MD 20014
Proc Natl Acad Sci USA; 73(5):1655-1659 1976

The resting state of the cell cycle was compared in normal and simian virus 40-transformed Chinese hamster lung cells. Normal cells deprived of amino acids or serum factors entered a resting state, whereas cells transformed by wild-type virus did not. The ability to enter a resting state was temperature-sensitive (ts) in cells transformed by a tsA mutant of the virus. When complete medium was added to resting cells, the length of time until the onset of DNA synthesis often exceeded the length of G1 in growing cells. The length of this interval depended upon the conditions used to arrest cell growth, but transferring cultures from medium depleted of one factor to medium depleted of a second factor never led to a round of DNA synthesis. DNA synthesis did not resume rapidly when a resting culture of cells transformed by tsA mutant was transferred to permissive temperature in suboptimal medium. A model is proposed in which cells in suboptimal conditions leave the cell cycle and traverse a branch pathway to enter the resting state. (24 refs)

280. TEMPERATURE-DEPENDENT FORMATION OF DIMERS AND OLIGOMERS OF MITOCHONDRIAL DNA IN CELLS TRANSFORMED BY A THERMOSENSITIVE MUTANT OF ROUS SARCOMA VIRUS (MEETING ABSTRACT).

Nass MM

Sch. Med., U. Pennsylvania, Philadelphia, PA
Proc Natl Acad Sci USA; 70(12):3739-3743 1973

In chick-embryo fibroblasts infected by T5, a temperature-sensitive mutant of Schmidt-Ruppin Rous sarcoma virus, the level of catenated dimeric and oligomeric mitochondrial DNA is temperature-dependent and correlates with the phenotypic manifestation of transformation. At the permissive temperature (36°C), where transformation is expressed, the elevated (2- or 3-fold) oligomeric level characteristic of cells transformed by the wild-type Rous sarcoma virus was observed, but at the nonpermissive temperature (41°C) at which cells appear normal and behave normally, the oligomer level was characteristic of uninfected cells. Temperature shifts from 41 to 36°C and vice versa resulted in phenotypic reversion and reversals of the respective levels of multiple-length RNA. Cellular growth rates were not altered. Treatment of control cells with cycloheximide resulted in a 5-fold increase in oligomeric DNA, whereas exposure to 9-beta-D-arabinofuranosyladenine had little effect. With both inhibitors, nuclear but not mitochondrial DNA synthesis was inhibited. A conformational and/or functional alteration in the mitochondrial membrane of a malignant cell might affect the attachment site of mtDNA and DNA replication. It is also possible that a protein factor or enzyme(s) involved in the replication of mtDNA is manufactured in the cytoplasm and then transferred into the organelles. The inhibition of cytoplasmic protein synthesis by cycloheximide might then lead to a deficiency of such enzyme(s).

281. TUMORIGENICITY OF VIRUS-TRANSFORMED CELLS IN NUDE MICE IS CORRELATED SPECIFICALLY WITH ANCHORAGE INDEPENDENT GROWTH IN VITRO (MEETING ABSTRACT).

Shin SI, Freedman VH, Risser R, Pollack R

Albert Einstein Coll. Medicine, Bronx, NY 10461
Proc Natl Acad Sci USA; 72(11):4435-4439 1975

Clonal isolates of mouse 3T3 cells and primary rat embryo cells, harvested nonselectively after infection by Simian virus 40 (SV40), were tested for tumorigenicity in the immune-deficient nude mice (a plus/nu stock on a BALB/c background). The cellular growth properties in vitro associated with neoplastic growth in vivo were observed. In addition, mouse 3T3 cells transformed by murine sarcoma virus (MuSV, Kirsten strain), and revertants isolated from cells fully transformed by either SV40 or MuSV were also studied. The single cellular property consistently associated with tumorigenicity in nude mice is the acquisition by virus-transformed cells of the ability to proliferate in vitro in the absence of anchorage. Anchorage-independent growth was measured by colony-forming ability in methylcellulose. Other cellular parameters of virus-induced transformation, such as lack of sensitivity to high cell density and the capacity to grow in low serum concentration, were dissociable from cellular tumorigenicity. Essentially identical results resulted from analogous experiments with four rat-embryo-cell clones. Specific selection in vivo for tumorigenic cells from anchorage-dependent mouse cells resulted in the isolation of anchorage-independent cells. Conversely, a single-step selection in vitro for anchorage-independent cells from nontumorigenic cells resulted in a simultaneous selection of highly tumorigenic subclones.

282. MODIFICATION OF THE PHENOTYPE OF MURINE SARCOMA VIRUS-TRANSFORMED CELLS BY SODIUM BUTYRATE. EFFECT ON MORPHOLOGY AND CYTOSKELETAL ELEMENTS.

Allenburg BC, Via DP, Steiner SH

Dept. Virology and Epidemiology, Baylor Coll. Medicine, Houston, TX 77030
Exp Cell Res; 102(2):223-231 1976

The effects on morphology and cytoskeletal elements of the modification of the phenotype of murine sarcoma virus (MSV)-transformed cells by sodium butyrate were studied. Normal rat kidney (NRK) cells transformed by and producing MSV and leukemia viruses NRK (MSV-MLV) were used. The cells could be cultured indefinitely in medium containing up to 2 mM butyrate. The growth rate declined during the first three to five passages in butyrate medium, but it was relatively constant thereafter. The cells began to extend cytoplasmic processes within 12 hr. After 24 hr in butyrate, approx 25% of the cells exhibited smooth or occasionally branched processes. The number of cells with altered morphology increased with time until virtually all of the cells in the culture showed process formation and many had begun to flatten. Following exposure to butyrate for 24 hr, the cells reverted to a morphology typical of MSV-transformed cells within 24 hr after removal of butyrate. After prolonged passage in butyrate, five or six passages involving at least 20 cell doublings were required for reversion to the typical transformed phenotype. Exposure of the MSV-transformed cells to butyrate resulted in the appearance of cytoplasmic actin fibers that closely resembled those seen in uninfected NRK cells. The butyrate-grown cells frequently contained a complex of cytoplasmic microtubules that radiated in parallel array from the region of the cell nucleus. The presence of cytoplasmic microfilament bundles and microtubules was demonstrated near the bottom of a butyrate-grown cell. The growth of NRK (MSV-MLV) cells in

butyrate induced cellular alterations that counteracted transformation of NRK cells by MSV. (22 refs)

283. SERUM DEPENDENCE OF EXPRESSION OF THE TRANSFORMED PHENOTYPE: EXPERIMENTS WITH SUBLINE OF MOUSE L FIBROBLASTS ADAPTED TO GROWTH IN SERUM-FREE MEDIUM.

Bershadsky AD, Gelfand VI, Guelstein VI, Vasiliev JM, Gelfand IM

Cancer Research Centre, Academy of Medical Sciences, Moscow, USSR

Int J Cancer; 8(1):83-92 1976

Characteristics of LSF cells grown in serum-containing and serum-free medium were compared. LSF is a subline of the L-strain of mouse transformed fibroblasts adapted to continuous growth in serum-free medium. Proliferation of LSF cells in monolayer on solid substratum was almost similar in serum-containing and in serum-free media. However, several other characters were found to be altered by the addition of serum to the serum-free medium: ability of cells to form colonies in semi-solid medium increased considerably; agglutinability of cells by Concanavalin A increased; uptake of 2-deoxy-D-glucose by the cells increased considerably; ability of cells to metabolize benzo(a)pyrene was inhibited; cell morphology was altered and, in particular, the cells became less spread on the substratum, and density of microvilli on the cell surface increased. All these changes induced by serum were reversed by transfer of the cells back into serum-free medium. Thus, addition of serum increased the expression of a number of cellular traits characteristic of transformed phenotype, while in serum-free medium partial phenotypic reversion of transformation was observed. A possible role of serum in the expression of the transformed phenotype is discussed. It is pointed out that cell lines adapted to growth in serum-free medium provide an experimental system convenient for analysis of the effects of different serum components on the cell phenotype. (Author Abstract)

284. PROPERTIES AND KINETICS OF DEVELOPMENT OF ROUS SARCOMA VIRUS-INFECTED CELLS EVIDENCED BY METHYLENE BLUE STAINING.

Barlati S, Mignatti P, Kryceve C, Vigier P

Laboratorio di Genetica Biochimica ed Evoluzionistica del CNR, I-27100 Pavia, Italy

Intervirology; 6(1):25-31 1975/76

Three different kinds of areas of infected cells corresponding to different focus formation stages were evidenced by methylene blue (MB) staining in cultures of chick embryo (CE) fibroblasts infected at low multiplicity with the temperature-sensitive (ts) mutant of Rous sarcoma virus (RSV), FU19, which transforms these fibroblasts at 37°C but not at 41°C. The areas are: areas of MB-stainable cells with transformed phenotype (STP areas = foci); areas of MB-stainable cells with normal phenotype (SNP areas); and areas of MB-unstainable cells with normal phenotype (USNP areas). To correlate stages of transformation with cellular DNA and RNA synthesis and virus production, DNA and RNA synthesis and virus production were followed in these various stages at 37°C and at 41°C. When the cultures were shifted from 37°C to 41°C, virus production in the SNP and USNP areas which arise by phenotypic revisions of STP foci remained comparable to that of the latter foci. DNA and RNA synthesis, however, were markedly reduced in SNP and USNP areas; DNA synthesis fell to the level of uninfected cells, while RNA synthesis remained somewhat higher. Comparison of the kinetics of development of SNP and STP areas in cultures infected with FU19 and with the parental virus SR4 confirmed that SNP areas are precursors of STP areas; this passage occurs at a slower rate in cultures infected with FU19.

285. TRANSFORMATION-ENHANCING FACTOR(S) PRODUCED BY VIRUS-TRANSFORMED AND ESTABLISHED CELLS.

Kryceve C, Vigier P, Barlati S

Institut du Radium (Biologie), Faculté des Sciences, 91405 Orsay, France

Int J Cancer; 17(3):370-379 1976

Chick embryo fibroblasts (CEF) and hamster BHK21 cells transformed by the Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV) release into the culture medium a factor or factors which enhance two- to seven-fold the formation of transformed foci by chick embryo fibroblasts infected with the Bryan strain of RSV (B-RSV). The factor(s) also increase the number of foci failing to revert to normal phenotype at restrictive temperature (41°C) in cultures infected with a temperature-sensitive mutant (FU-19) of SR-RSV which is defective for transformation. The factor(s) is also produced by BHK21 cells transformed by other tumor viruses and by BHK21 cells passaged for a long time, but not by normal CEF, CEF transformed by B-RSV, CEF infected by FU-19 at 41°C, normal hamster embryo fibroblasts, established but density-inhibited mouse fibroblasts, or BHK21 cells of early passages. The relative enhancement of the number of B-RSV foci can be more than 100-fold when the medium contains fetal calf serum, which suppresses focus formation in controls. The focus-enhancing factor(s) appears to act after infection and has been termed, operationally, transformation-enhancing factor(s) (TEF). The factor produced by RS2/3 cells which enhances the formation of B-RSV foci is non-dialyzable and thermolabile, and is presumably a protein. Its molecular weight is between 10^5 and 2×10^5 daltons.

F. Tumor Antigens of Transformed Cell Properties

286. ALTERNATE CHANGES OF SURFACE ANTIGEN(S) IN ADENOVIRUS TYPE 12-TRANSFORMED AND TUMOR CELLS.

Nakajima S, Hamada C, Uetake H

Inst. Virus Res., Kyoto U., Japan

Jpn J Microbiol; 17(4):303-311 1973

Alternate changes of specific surface antigen(s) (S antigen) were examined in transformed and tumor cells induced by human adenovirus type 12. All of the hamster and mouse cells transformed in vitro showed ringform membrane fluorescence staining by anti-S antigen rabbit sera, whereas tumor cells, either induced by the virus in vivo or produced by inoculation with the S(+) transformed cells, did not show any fluorescence. When the S(-) tumor cells were serially subcultured in vitro, all of them converted to S(+) cells, although more than ten subcultures were necessary. For the S(+) cells to form tumors in hamsters about ten times as many cells were necessary as the S(-) cells. This difference became greater when tumor formation was tested in preimmunized hamsters, while little, if any, when tested in X-irradiated hamsters. In addition, immunogenicity of the S(+) cells was suggested to be higher than that of the S(-) cells. These findings indicate that the S(+) cells are more immunosensitive and immunogenic than the S(-) cells, and that in vivo conversion from S(+) to S(-) may be due to selection of S(-) mutant cells. In vitro conversion from S(-) to S(+) was also suggested to be due to the appearance of S(+) mutant cells.

287. CELL SURFACE ANTIGEN INDUCED BY AVIAN TUMOR VIRUSES IN HAMSTER CELLS TRANSFORMED BY A TEMPERATURE-SENSITIVE MUTANT OF ROUS SARCOMA VIRUS.

Aupoix MC, Biquard JM, Cachard A
Virol. Unit., INSERM, Lyons, France
Int J Cancer; 14(5):611-616 1974

A cell surface antigen induced by avian tumor viruses in BHK21/13 hamster cells transformed by a temperature sensitive mutant of Rous sarcoma virus was reported. The hamster cells were transformed at the permissive temperature (37°C) by the mutant FU 19 of the Schmidt-Ruppin strain. They exhibited a temperature-dependent expression of virus-induced surface antigen as assayed by mixed hemadsorption test. This temperature dependence of virus-induced surface antigen was not observed in cells transformed by the wild type virus, SR4, although the number of positive cells was reduced at the restrictive temperature (41°C) when compared to cells incubated at the permissive temperature. The temperature dependency suggests that the appearance of virus-induced surface antigen depends on the expression of viral genes and, in particular, on the production of some specific virus coded proteins. The fact that the expression of virus-induced surface antigen is also variable in the wild type transformed clone (RS2/3) also suggests that a cellular function could be involved in the regulation of virus-induced surface antigen synthesis.

288. DISTRIBUTION OF FIBROBLAST SURFACE ANTIGEN. ASSOCIATION WITH FIBRILLAR STRUCTURES OF NORMAL CELLS AND LOSS UPON VIRAL TRANSFORMATION.

Wartiovaara J, Linder E, Ruoslahti E, Vaheri A
Dept. Third Pathol., Univ. Helsinki, Finland
J Exp Med; 140(6):1522-1533 1974

The localization of a cell type-specific, soluble surface antigen (SFA) previously detected in chick embryo fibroblasts was studied by immunofluorescence and scanning electron microscopy. The antigen was unevenly distributed on cultured chick fibroblasts; it was localized to membrane processes and ridges that varied from 50-200 nm in diameter. The processes extended from the periphery of the cells to the substratum or to other cells. Trypsin treatment removed all detectable SFA, but the antigen reappeared within one hr after trypsin-treated cells were reseeded. Its reappearance correlated with the restoration of membrane processes. Fibroblasts transformed by Rous sarcoma virus (RSV), showed loss of all or most SFA. In cells transformed without subcultivation and trypsinization, a fibrillar extracellular network of SFA was detectable even though the cells themselves were negative in immunofluorescence. When fibroblasts infected by a temperature-sensitive RSV mutant were transferred to the nonpermissive temperature for transformation, new SFA was detected within two hr. These data suggest that loss of SFA molecules in fibrillar cell surface structures may be critical in altered growth control and malignant transformation.

289. DIFFERENTIAL INDUCTION OF TUMOUR ANTIGENS BY TRANSFORMATION-DEFECTIVE VIRUS MUTANTS

Kurth R
Imperial Cancer Res. Fund Lab., P.O. Box 123, Lincoln's Inn Fields, London, WC2A3PX, England
J Gen Virol; 28(2):167-177 1975

Normal rat (outbred Osborn-Mendel strain) kidney cells infected by a variety of transformation-defective temperature-sensitive avian leukosis sarcoma virus mutants were tested for the expression of transformation characteristics at permissive and restrictive temperatures. Morphology, growth behavior

and agglutinability by concanavalin A corresponded fully to the phenotype of the infected cells, at permissive temperature the cells resembled wild type virus transformed cells, whereas when grown under restrictive conditions they became virtually indistinguishable from normal cells. The quantitative expression of allo- or xenogeneic cell surface antigens was not significantly affected by the phenotype of the cells. Two out of the five tested mutants induced tumor antigens in the expected temperature-dependent manner, whereas the other three mutants were able to induce tumor-specific cell surface antigens even in the revertant cells cultured at the restrictive temperature. These findings extend previous results about tumor antigen induction in mutant-infected cells of the natural host, the chicken embryo fibroblasts. The value of transformation-defective tumor antigen-positive mutants for vaccination purposes is discussed.

290. THE EFFECT OF TRANSFORMATION-DEFECTIVE AVIAN ONCORNAVIRUS MUTANTS ON TUMOR ANTIGEN EXPRESSION.

Kurth R, Wyke JA, Friis RR, Rohrschneider L, Bauer H
Friedrich Miescher-Laboratorium, Max Planck-Insts, D74 Tübingen, West Germany
J Supramol Struct; 4(1):133-139 1976

The effect of transformation-defective avian sarcoma virus mutants on the phenotype and cell surface membrane of infected avian and mammalian cells was investigated, with particular emphasis on the mode of expression of tumor specific surface antigen (TSSA) in mutant-infected, phenotypically normal cells. TSSA was detected on mutant infected cells by a paired radioactively labeled antibody technique in which the same quantities of normal and anti-TSSA chicken IgG are labeled with either ^{125} I or ^{131} I, and their absorption to normal and mutant-infected target cells is measured. All of the tested nontransforming deletion mutants and the majority of the temperature-sensitive (ts) mutants were unable to induce tumor antigens in phenotypically untransformed cells. Three temperature-sensitive mutants (tsLA23, tsLA24, and tsLA31), however, were able to support the expression of TSSA even at restrictive temperatures, when cells otherwise exhibited a normal phenotype. Two hypotheses are presented to explain this apparent contradiction: (a) the three mutants possess a late defect and virus replication at 41°C remains unaltered; most but not all transforming proteins are synthesized, and the transformation process is blocked at a later metabolic stage than TSSA expression; or (b) TSSA may be the ts gene product and under restrictive conditions may still be synthesized, but in a functionally inactive form, and inserted into the membrane. It is suggested that this hypothesis is likely if TSSA turns out to represent a tumor cell surface associated enzyme.

291. CONTINUED PRESENCE OF SIMILAR TRANSFORMATION-ASSOCIATED ANTIGENS RELATED TO MURINE ONCORNAVIRUS PROTEINS IN TRANSFORMED CELLS, MORPHOLOGICAL REVERTANTS, AND CELLS RESTRICTED IN THE EXPRESSION OF TRANSFORMATION.

Rieber M, Bacalao J, Rieber M
Center of Microbiology and Cell Biology, Instituto Venezolano de Investigaciones Científicas, Apartado 1827, Caracas, Venezuela
Cancer Res; 37(4):1170-1174 1977

The possible alteration of transformation-related antigens in cells that revert to a normal phenotype but that continue to retain the viral genome, has been investigated in [^{3} H]glucosamine-labeled extracts of rat cells exhibiting a reversible temperature-dependent restriction in the expression of transformation and in a comparison of a morphologically altered mouse cell transformed by the Kirsten sarcoma virus

with a flat revertant mouse cell derived from the morphologically transformed cells. With the use of normal goat serum in the presence of dibutyl cyclic 3':5'-adenosine monophosphate, some differences became obvious in the rat cells restricted in the expression of transformation. However, use of specific antiserum to murine leukemia virus revealed in every case the presence of major components that exhibited an electrophoretic mobility corresponding to about 100,000 daltons both in the parent and revertant mouse cells and in the rat cells exhibiting either untransformed or transformed growth properties. The glycoprotein components detected only by the immune serum may represent a cellular macromolecule antigenically related to an interspecies C-type viral species whose concentration is increased in transformed cells. (10 Refs) (Author Abstract)

G. Other Alterations of Transformed Cell Properties

292. AN ENZYMATIC FUNCTION ASSOCIATED WITH TRANSFORMATION OF FIBROBLASTS BY ONCOGENIC VIRUSES. I. CHICK EMBRYO FIBROBLAST CULTURES TRANSFORMED BY AVIAN RNA TUMOR VIRUSES.

Unkles JC, Tobia A, Ossowski L, Quigley JP, Rifkin DB, Reich E
Rockefeller U., New York, NY
J Exp Med; 137(1):85-111 1973

Rous sarcoma virus (RSV)-transformed chick embryo fibroblasts (CEF), unlike normal, untransformed CEF, showed fibrinolysin activity, as determined by the ability to solubilize 125I-fibrin *in vitro*. A similarly enhanced fibrinolytic activity was observed in CEF cells transformed by a number of different transforming viruses. However, cultures infected with nontransforming viruses, with a temperature-sensitive RSV mutant at permissive temperatures, or with various cytotoxic viruses, failed to show significant fibrinolytic activity. The appearance of fibrinolytic activity in CEF cells infected with the RSV mutant following a shift to permissive temperatures was dependent on RNA and protein synthesis, but not on DNA synthesis. Of all sera tested, those of chicken and monkey origin were most active in promoting fibrinolysis by transformed cells. Other sera, including fetal bovine, adult bovine, turkey, dog and guinea pig, were considerably less effective and some (e.g. fetal bovine) contained a fibrinolysin inhibitor. Normal CEF did not secrete fibrinolysin inhibitor nor was one found in the sera from normal birds. Sera from sarcoma-bearing chickens did, however, contain an inhibitor of fibrinolysin.

293. EFFECT OF LOSS OF THYMIDINE KINASE ACTIVITY ON THE TUMORIGENICITY OF CLONES OF SV40-TRANSFORMED HAMSTER CELLS (MEETING ABSTRACT).

Rothschild H, Black PH
Massachusetts Gen. Hosp., Boston, MA
Proc Natl Acad Sci USA; 67(2):1042-1049 1970

Characteristics of cells (derived from transplantable SV40-transformed hamster kidney cells) deficient in thymidine kinase (TK-) were studied. Wild type cells (TK+) incorporated 100 times as much ³H-thymidine (11.0-23.0 x 10³ cpm/10⁶ cells). TK+ cells had a large variation in chromosome number (53-114 chromosomes) while TK- cells (T8 BU 1-1) had relatively constant modal numbers (52-54 chromosomes). Tumors initiated by TK+ cells appeared in 95% of the animals 2-8 wk after inoculation, while only 1 tumor was observed in hamsters inoculated with TK- cells by the 8th wk. Cells harvested from tumors induced with TK- cells and revertant cell lines (spontaneous or chemically mutated) obtained from TK- cells showed thymidine kinase activity to

be intermediate between that of the wild type and the enzyme-deficient cell lines. The thymidine kinase salvage pathway enzyme may play a rate-limiting role in tumorigenesis.

294. ALTERATIONS IN GLUCOSE METABOLISM IN CHICK EMBRYO CELLS TRANSFORMED BY ROUS SARCOMA VIRUS: TRANSFORMATION-SPECIFIC CHANGES IN THE ACTIVITIES OF KEY ENZYMES OF THE GLYCOLYTIC AND HEXOSE MONOPHOSPHATE SHUNT PATHWAYS.

Singh M, Singh VN, August JT, Horecker BL
Div. Biol. Sci., Albert Einstein Coll. Med., Bronx, NY
Arch Biochem Biophys; 165(1):240-246 1974

Primary cell cultures of 10-day chick embryos were infected with either wild type Rous sarcoma virus (RSV), or the temperature-sensitive mutant (Ts-68), of the Schmidt-Ruppin strain to observe the effects of transformation on the activity of key enzymes involved in glucose metabolism. In 4-day-old secondary cultures the activities of the three rate-limiting glycolytic enzymes, hexokinase, phosphofructokinase and pyruvate kinase, as well as that of lactate dehydrogenase, were increased by about two fold. For enzymes of the hexose monophosphate shunt pathway, glucose-6-P-dehydrogenase activity was increased significantly while 6-P-glucanate dehydrogenase activity was unchanged. Hexokinase activity, in transformed as compared to normal cell fractions, was markedly increased in the particulate, i.e. bound, fraction but was unchanged in the soluble fraction. To differentiate between activity changes due to transformation, and those due to infection, the Ts-38 mutant (which is normal for infection and replication but temperature sensitive for transformation) was used. The results showed that for hexokinase, phosphofructokinase, pyruvate kinase and glucose-6-P-dehydrogenase increased activities and increased sugar uptake were associated specifically with the process of transformation; lactate dehydrogenase was different, i.e. infected cells had increased activity at both the permissive and nonpermissive temperatures. The increased enzyme activities of the transformed cells apparently provide a mechanism for increased flux of glycolysis and glucose transport. The increased rates of glycolysis in transformed cells appear to be genuinely related to the increase in activities of the key enzymes studied.

295. SPECIFIC INCREASE IN POLYAMINE LEVELS IN CHICK EMBRYO CELLS TRANSFORMED BY ROUS SARCOMA VIRUS.

Don S, Wiener H, Bachrach U
Heb. Univ. Hadassah Med. Sch., Jerusalem, Israel
Cancer Res; 35(1):194-198 1975

To demonstrate the relation between cellular polyamines and transformation with oncogenic viruses, chick embryo fibroblasts and chorioallantoic membranes were infected with wild type (SR-17A) or temperature-sensitive (T5) Rous sarcoma virus (RSV) mutants at permissive (37°C) or nonpermissive (42°C) temperature. Cells were analyzed for polyamines, and the results were compared to polyamine content of chorioallantoic membranes infected with nononcogenic viruses (influenza strain PR8, Newcastle disease velogenic strain Sp and lentogenic strain K, and vaccinia virus strain WR). Primary chick embryo fibroblasts were infected with RSV (0.2 ml) on day 1. Subcultures were made on day 5, and secondary cells were infected with RSV (1.0 ml) on day 6. Growth medium was first changed at least three days after cells were seeded and once during each subsequent day. The incubation temperature of some cultures was shifted on day 10. All groups were analyzed for polyamines on day 11. The chorioallantoic membranes of 12- to 13-day old chick embryos were infected with RSV (0.05-0.1 ml). Membrane fragments were collected six days later, washed in 0.85% NaCl, and

analyzed for polyamines. Only putrescine accumulated in chick embryo fibroblasts transformed by RSV at 37 C. The nononcogenic viruses had no effect on the polyamine content of chorioallantoic membranes. Transformation of membranes by both the SR-17A and T5 strains of RSV at 37 C caused a 2- to 4-fold increase in cellular spermidine and putrescine content. At 42 C, RSV strain T5 did not alter cellular morphology or polyamine content. These data suggest that if polyamines are involved in neoplastic growth, then polyamine analogs may present a new series of potential therapeutic agents.

296. POLYAMINE METABOLISM IN NORMAL AND IN VIRUS-TRANSFORMED CHICK EMBRYO FIBROBLASTS.

Don S. Bachrach U

Hebrew Univ.-Hadassah Medical Sch., Jerusalem, Israel
Cancer Res: 35(12):3618-3622 1975

The effect of transformation by Rous sarcoma virus on the ornithine decarboxylase levels of chick embryo fibroblasts was studied. Infection with Rous sarcoma virus resulted in increased ornithine decarboxylase activity (which preceded morphological alterations by at least 24 hr). Ornithine decarboxylase levels of normal controls, unlike those in Rous sarcoma virus-infected cells, declined with age; the enzyme activity of transformed cells was increased 20-fold 5 days postinfection. Infection with a temperature-sensitive mutant of Rous sarcoma virus, T5, stimulated ornithine decarboxylase activity of chick embryo fibroblasts at the permissive (37 C) but not at the nonpermissive (42 C) temperature. Ornithine decarboxylase activity of T5-infected cells (shifted from 42 C to 37 C); increased within 2 hr and reached the levels of wild-type, Schmidt-Ruppin-17A-transformed cells in 11 hr. When T5-transformed fibroblasts were shifted to the nonpermissive temperature, ornithine decarboxylase activity decreased fivefold within 4 hr and paralleled the levels of normal controls in 11 hr. Shifting of temperatures of incubation caused comparable alterations in cellular putrescine.

297. CHANGES IN LEUCINE AMINOTRANSFERASE ISOZYMES BY VIRAL TRANSFORMATION AND ITS CORRELATION WITH THE ISOZYME CHANGES OCCURRING DURING DIFFERENTIATION.

Roth SL, Delotto R, Kaji A

Dept of Microbiology, Sch of Med, Univ of Pennsylvania, Philadelphia, PA 19174
Cancer Res: 37(4):1147-1153 1977

The isozyme pattern of leucine aminotransferase (EC 2.6.1.6) in various cell lines and their viral transformed derivatives were examined. The Wistar 3C rat liver cell line was found to contain only isozyme I, while its simian virus 40-transformed counterpart had isozyme III in addition to isozyme I. A spontaneously transformed late passage clone of these liver cells was also found to have acquired isozyme III. Polyoma virus-transformed baby hamster kidney cells were also found to contain a greater predominance of isozyme III than their normal untransformed counterpart. Examination of the isozymes in a cloned normal rat kidney cell line transformed by a mutant of Rous sarcoma virus which is temperature-sensitive for transformation indicated that, in fact, such an isozyme change does correlate with transformation. When grown at 36 degrees these cells contained predominantly isozyme III; however, upon reacquiring normal morphology and lowered glucose transport activity when grown at 40 degrees, their isozyme pattern was now found to be changed and consisted predominantly of isozyme I, as is found in normal adult rat kidney tissue. Isozyme III was found to be present in neonatal kidney tissue of the rat and hamster, and its predominance in the virus-transformed normal rat and baby hamster kidney cells was interpreted as

indicative of the dedifferentiation of these cells upon viral transformation. A similar change of the isozyme pattern of leucine aminotransferase in chicken embryos during their development was observed, such that in 5-day-old embryos Form III was predominant, while in the more mature differentiated chicken embryo of Day 17, Form I was predominant. (37 Refs) (Author Abstract)

298. ROUS SARCOMA VIRUS ACTIVATES EMBRYONIC GLOBIN GENES IN CHICKEN FIBROBLASTS (MEETING ABSTRACT).

Groudine M, Weintraub H

Dept, Biochemical Sciences, Princeton Univ., Princeton, NJ 08540

Proc Natl Acad Sci USA: 72(11):4464-4468 1975

To determine whether a specific cellular gene, the globin gene, becomes activated after Rous sarcoma virus (RSV) transformation, globin complementary DNA (cDNA) made against globin messenger RNA (mRNA) from adult chicken reticulocytes was annealed with the total RNA from RSV-infected and uninfected chick embryo fibroblasts. The globin cDNA failed to hybridize to the total RNA extracted from uninfected fibroblasts, but RNA complementary to globin cDNA was detectable in amounts of 100-500 copies per cell in RSV-infected fibroblasts. No globin mRNA sequences were detectable in purified RSV RNA. When the globin cDNA probe was reacted with total RNA from fibroblasts infected with a transformation defective (td) RSV, no hybridization was detected, even though the mutant replicated and produced normal numbers of virus particles. In further experiments, chick embryonic RBC RNA was added to a hybridization mixture containing RNA from RSV-transformed fibroblasts and the cDNA probe. Only 71% of the probe was saturated, indicating that the globin sequences that are unique to the adult were not activated during RSV transformation of the chick fibroblasts. Several embryonic globin mRNAs may have been activated. The hybrids formed between the RNA from RSV-infected fibroblasts and the globin cDNA probe were stable. The data suggest a strict correlation between the presence of the 'one' gene (the viral gene which controls the expression of the phenotypic changes associated with RSV transformation), host cell transformation, and the activation of embryonic globin gene transcription.

299. TEMPERATURE-SENSITIVE EXPRESSION OF DIFFERENTIATION IN TRANSFORMED MYOBLASTS.

Fizman MY, Fuchs P

Dep. Biol., Massachusetts Inst. Technol., Cambridge, MA
Nature (Lond): 254(5499):429-431 1975

The in vitro transformation of chick embryo myogenic cells with a temperature-sensitive mutant of Rous sarcoma virus (RSV) is reported. Chick myoblasts derived from thigh muscles of 11-day-old chick embryos were infected with the Schmidt-Ruppin ts68 strain of RSV. In mock-infected chick embryo myoblasts, after five days in culture, most of the mononucleated cells were found replaced by long myotubes, each containing numerous nuclei. However, in the RSV-infected cultures, the number of myotubes found was reduced, and many aggregates of rounded cells, presumably transformed were found. A quantitative study revealed a change in the overall composition of the population after a shift from 36 C (permissive temperature) to 41 C (nonpermissive temperature); whereas at 36 C, 99% of the cells remained mononucleated; 60% became polynucleated at 41 C. RSV-transformed myoblasts could be isolated, and after serial passage, these myoblasts were prevented from further differentiation. The function was not lost, however, because a shift up to a nonpermissive temperature again revealed differentiation to form myotubes. The cells which did not fuse at 41 C could have been transformed fibroblasts, or

transformed myoblasts which had definitively lost their ability to differentiate. A striking difference was found between the myotubes appearing after infection and those formed after the shift to 41°C; the latter were found very stable and remained as long as myotubes in an uninfected culture. The results indicate that it was possible to isolate transformed myoblasts in which the expression of differentiation is under viral gene control.

300. EFFECT OF ONCOGENIC VIRUS ON MUSCLE DIFFERENTIATION.

Holtzer H, Biehl J, Yeoh G, Meganathan R, Kaji A
Departments of Anatomy and Microbiology, School of
Medicine, University of Pennsylvania, Philadelphia, PA
19174

Proc Natl Acad Sci USA; 72(10):4051-4055 1975

Chick muscle cultures infected with wild-type Rous sarcoma virus form myotubes, but these myotubes vacuolate and by day 6 most have degenerated, leaving only large numbers of transformed mononucleated, replicating cells. Muscle cultures infected with a temperature-sensitive mutant (TS) at permissive temperatures behave as cells infected with wild-type Rous sarcoma virus. TS-infected cells reared for 8 days at nonpermissive temperature form contracting myotubes plus large numbers of fibroblastic cells. If these cultures are lowered to permissive temperature, within 72 hr the myotubes vacuolate and degenerate, whereas the mononucleated cells transform. If replicating TS-transformed cells after 8 days at permissive temperature are shifted to nonpermissive temperature, within 72 hr many cells fuse and form contracting, post-mitotic myotubes. Creatine kinase (ATP:creatine N-phosphotransferase, EC 2.7.3.2) levels parallel the formation and degeneration of myotubes during these temperature shifts. If the viral transforming gene is expressed in the post-mitotic myotubes it is lethal, whereas it is not lethal if expressed in replicating precursor myogenic cells. The viral gene expression at permissive temperature blocks further myogenesis depending on the position of the cells in the myogenic program. The virus does not cancel the replicating, transformed myogenic cells' commitment to, or position in, the myogenic lineage. When the transforming action of the virus is suppressed, the normal myogenic program resumes. (Author Abstract)

301. ACCUMULATION OF WATER DURING TRANSFORMATION OF CELLS BY AN AVIAN SARCOMA VIRUS.

Bader JP, Ray DA, Brown NR
Natl. Cancer Inst., Bethesda, MD
Cell (Camb); 3(3):307-313 1974

Potential size-related changes in the biophysical parameters of virus-transformed cells were investigated using cultured chick embryo (CE) cells transformed by the Bryan "high titer" strain of the Rous sarcoma virus (RSV-BH). The transformed cells were larger than nontransformed CE cells, as determined by direct measurements of diameter and volume. While the observed general increase in cell mass was insufficient to fully account for the increase in size, the RSV-BH-transformed cells were found to contain a greater proportion of water than the untransformed cells; this was reflected in a decrease in cellular density in the former. The observed changes in size, water content, and density were not found in cells infected with the Schmidt-Ruppin strain of avian sarcoma virus. CE cells infected with a mutant of RSV-BH were morphologically transformed at 37°C but not at 41°C; when shifted from 41 to 37°C, they became larger, accumulated more water, and banded higher in density gradients. These changes occurred without a requirement for new RNA or protein synthesis. The increase in the cellular water content may be an early event leading to changes in transcription and translation

and culminating in malignancy in the RSV-BH-transformed cells.

302. TRANSFORMATION OF CELLS BY ROUS SARCOMA VIRUS: CYTOPLASMIC VACUOLIZATION.

Bader AV, Bader JP
Natl. Cancer Inst., Bethesda, MD 20014
J Cell Physiol; 87(1):33-46 1976

Chick embryo cells transformed by the Bryan "high titer" strain of Rous sarcoma virus (RSV-BH) were heavily vacuolated. A variety of microscopic techniques were used in demonstrating that the vacuoles were cytoplasmic, bounded by membrane, and were composed largely of water. Proteins, lipids, glycoproteins, glycolipids, glycosaminoglycans, glycogen, and nucleic acids were undetectable in the vacuoles. Physiological requirements for development of the vacuoles and reversal of vacuolization, were examined in cells infected with a virus mutant, RSV-BH-Ta, which induced reversible temperature-dependent transformation. NaI was the only component of the cell culture medium found essential for both the development and reversal of vacuoles. Glucose depletion or dinitrophenol treatment inhibited vacuolization (suggesting a possible energy requirement in the vacuolization process). Ouabain enhanced vacuolization. Glucosamine and mannosamine prevented the disappearance of vacuoles. Cellular vacuolization could be a normal physiological response to an increase in water and NaI.

VII. REVIEWS AND GENERAL ARTICLES

303. TEMPERATURE-SENSITIVE MUTATIONS IN ANIMAL CELLS.

Basilico C
Dept. Pathology, New York Univ. Sch. Medicine, New
York, NY
Adv Cancer Res; 24:223-266 1977

Temperature-sensitive (ts) mutants of animal cells can be obtained with reasonable frequency. The mutants are stable and homogeneous, with a low reversion frequency. A review is presented of the following aspects of ts mutations: methods of induction and selection, frequency and general behavior, characterization, and genetic analysis. The characteristics of some ts growth mutants are tabulated. Particular discussion is made of cell lines with ts mutations that affect the expression of specialized functions and in vitro transformation properties. Ts mutations are recessive; the development of methods provoking monosomies or chromosomal deletions should thus be of great advantage in increasing the yield of ts mutants. (90 Refs)

304. THE CHICK FIBROBLAST CELL SURFACE AFTER TRANSFORMATION BY ROUS SARCOMA VIRUS. (PP. 1173-1180)

Robbins PW, Wickus GW, Branton PE, Gaffney BJ, Hirschberg CB, Fuchs P, Blumberg PM
Tumor Viruses Cold Spring Harbor Symposia on
Quantitative Biology, Cold Spring Harbor Lab, Cold
Spring Harbor, NY, vol. 39, 1975

Secondary chick embryo fibroblasts transformed by the ts 68 mutant of the Schmidt-Rupin strain of Rous sarcoma virus (RSV) were used to compare lipid fluidity, cellular proteins, and actin in normal and transformed cell membranes. The transformed fibroblasts have a normal morphology at 41°C but rapidly assume a transformed morphology at 36°C. Although ts 68-infected cells at 36 and 41°C may be slightly more rigid than the corresponding normal cells, there was no difference in fluidity that could be clearly associated with transformation. Similarly, there were no consistent differences in the fluid lipid regions of normal and transformed plasma

membranes. The plasma membranes of transformed cells also showed decreased levels of three specific cellular proteins: omega, delta, and zeta. Omega, a polypeptide with a molecular weight of about 206,000, ceased to be synthesized or began to degrade almost immediately on shift from 41 to 36 C. Delta, which appears to be a glycoprotein, was synthesized at control levels for the first 3 hr after the temperature shift in both infected and uninfected cells, after which the synthesis rate dropped (to approximately 10%-20% of the 41 C rate in uninfected cells). A glycoprotein of about 70,000 daltons was also present in higher amounts in infected cells at 41 C than at 36 C; this may represent an accumulation of viral protein. The zeta polypeptide is extracellular, exhibits protease sensitivity, decreases in concentration in transformed cells, and may be related to a procollagen type of molecule. Its degradation may not represent a fundamental requirement for transformed cell morphology. RSV transformation appeared to reduce the amount of "polymerized" actin and increase "monomeric" actin, but had no effect on the total quantity of actin in the plasma membrane. Alterations in actin biochemistry may be involved in the production of some of the transformed cell phenotypic properties.

305. CHARACTERISTICS OF CELLS TRANSFORMED BY ROUS SARCOMA VIRUS. (PP. 303-313)

Bader JP, Marciani DJ

Fundamental Aspects of Neoplasia. Gottlieb AA, Plescia OJ, Bishop DH, ed. New York, Springer-Verlag, 1975.

Transformation by temperature-sensitive (ts) mutants of Rous sarcoma virus, the biophysical and metabolic changes accompanying transformation, vacuolization, and the effects of cell surface membrane proteins are reviewed in an attempt to identify a molecule initially responsible for transformation. Cells infected with the mutant ts virus, RSV-BH-Ta, behave in a normal fashion at 41 C and transform after a temperature shift to 37 C. The change in RSV-BH-Ta cells occurs without any requirement for new DNA, RNA, or protein synthesis. Morphologic changes observed microscopically in transforming cells include rounding, intense vacuolization, and an increase in size due to increased intracellular water. None of the metabolizable components of the cell culture medium, including serum, amino acids, vitamins, or glucose, are essential for vacuolization and no requirement for Ca^{+2} , Po_4^{+3} , or HCO_3^- has been shown. Removal of either K^{+} or Mg^{+2} partially prevents vacuolization; substitution of Li^{+} for Na^{+} prevents it completely. Metabolic differences between chicken embryo cells and RSV-BH transformed cells include a fivefold increase in the uptake of glucose and glucose analogs from the medium and a twofold increase in the uptake of galactose, leucine, uridine, and thymidine. Hyaluronic acid synthesis increases 5 to 10 times within 6 hr after RSV-BH-Ta cells are shifted from 41 to 37 C. These increases are prevented by actinomycin D or cycloheximide but not by cytosine arabinoside, indicating that synthesis of new RNA and protein, but not DNA, is required. None of these activities, therefore, is responsible for the increased accumulation of intracellular water or vacuolization, since these changes require neither RNA nor protein synthesis. Analysis of the protein constituents of cell surface membranes by electrophoresis showed a decrease in high molecular weight proteins in transformed RSV-BH-Ta cells, suggesting that protein loss is a result rather than a consequence of transformation. It is concluded that a molecule involved in the regulation of water uptake or elimination is initially responsible for the physiologic manifestation of malignancy and that intracellular Na^{+} and K^{+} concentrations are also affected in this initial transformation step. (14 refs)

306. GENETIC ASPECTS OF ROUS SARCOMA VIRUS INFECTION.

Hanafusa H, Kawai S, Hanafusa T, Scheele CM, Metroka C

Dept. Viral Oncol., Public Health Res. Inst. City New York, NY

Collect Pap Annu Symp Fundam Cancer Res; 25:206-215 1974

The genetic analysis of Rous sarcoma virus (RSV) infection aims at elucidation of the mechanism of cell transformation. Defective functions of Bryan RSV are reviewed and recent studies of Schmidt-Ruppin RSV mutants which are temperature sensitive in cell transformation are described. In addition to the infectious helper virus previously known to be essential to replication of Bryan RSV, another helper, unable to mature into infectious helper virus by itself, is contributed by normal chicken cells. This chick cell-associated helper factor (chf) is genetically controlled, and every cell from a single embryo is equally either positive or negative for chf. Positive cells produce infectious virus, RSV(+), while negative cells produce only noninfectious virus, RSV(-). SDA-gel electrophoretic analysis showed that Bryan RSV possesses multiple envelope glycoprotein moieties, while leukemia or helper independent sarcoma viruses contain a single moiety with low electrophoretic mobility. This slow glycoprotein was absent from Bryan RSV(-). The work thus provided physical evidence for defectiveness. In the studies on Schmidt-Ruppin RSV temperature-sensitive mutants, which cause cells to be reversibly transformed at 37 C and reversibly return transformed cells to normal at 41 C, complementation analyses were carried out whereby tests were made for properties of recombinants obtained by means of mixed infection with SR-RSV (subgroup A) and RAV-2 (subgroup B). The mixed infection produced SR-RSV with subgroup B specificity. Using the same procedure, three temperature-sensitive mutants were converted from subgroup A to B, maintaining the temperature-sensitivity. Results obtained with different combinations of the mutants suggested that virus may have more than two cistrons for temperature-sensitive proteins essential for cell transformation, that the protein consists of two subunits, or that the two temperature-sensitive components could be the same subunit of an enzyme, which is composed of more than two molecules of the subunit, with a disorder of amino acid sequence at different locations.

307. A STUDY OF THE ONCOGENIC AND INFECTIOUS FUNCTIONS OF ROUS SARCOMA VIRUS.

Golde A

Fac. Sci. Orsay, France

Bull Cancer (Paris); 57(2):195-216 1970

Oncogenic action and reproduction capability of Rous virus in vitro were investigated on chick embryo fibroblast cultures with various mutants of the Schmidt-Ruppin strain by varying the conditions of cell culture, promoting one or the other of the investigated functions. Two defective mutants of the Schmidt-Ruppin strain were obtained by exposure to 3000 kr or 6 Co emitting gamma-radiations; one mutant had oncogenic properties but was unable to reproduce itself within the transformed cell culture, and the other was a noncarcinogenic mutant capable of reproduction. The neoplastic nature of the cells transformed by the oncogenic mutant was confirmed by their transplantability into eggs with 10 day-embryos; these cells did not release virions and were referred to as NP cells. The non-oncogenic mutant was referred to as NT gamma mutant and produced no morphological alterations in chick embryo fibroblasts. However, these virions contained a group specific (gs) antigen characteristic for avian oncogenic viruses. The occurrence of

these 2 different mutants suggested that oncogenicity and reproduction were coded by different independent portions of the viral genome; it was postulated that the viral RNA molecule could be constituted of several independent subunits. Variations of culture conditions indicated that the infection and transformation efficacy of Schmidt-Ruppin and Bryan mutants was not cell-age dependent. These properties seemed to be affected by the number of monocellular layers (degree of confluence) at the moment of infection. A cellular density of 3 monolayers appeared to be the threshold when cell transformation would no longer occur, although infection and virion production were still possible. The factor of cellular density apparently did not affect infectivity and virion productivity but did inhibit the reading of the viral information needed to induce cell transformation.

308. MUTANTS OF ROUS SARCOMA VIRUS.

Vigier P

Inst. Radium Biol., Orsay, France

Bull Cancer (Paris); 59(1):21-32 1972

The Rous sarcoma virus (RSV) is reviewed, including the nature of the viral genome, information for replication, and classes of mutants. It is postulated that its genome is replicated by DNA which directs the synthesis of RNA replicates for the production of the virus in permissive cells. The RSV carries transforming information which is apparently the same for in vitro and in vivo cells. The RSV mutants may be classified as: morphologic mutants, determining the specific structure of the transformed cells; defective in their power to transform cells; and conditional mutants, which include, on the one hand, mutants that have lost their transforming capacity (but not the capacity for replication) at a high temperature, and on the other hand, mutants that have lost both capacities at a high temperature. It is suggested that the protein synthesis required for the production of the transformation, determines a modifiable alteration of the surface constituents of the cell, either in uncovering preexistent sites or in modifying their configuration or distribution.

309. RNA TUMOR VIRUS GENETICS 1975.

Vogt PK

Univ. Southern California, Sch. Medicine, Los Angeles, CA

Adv Pathobiol; 4:29-37 1976

The genetics of RNA tumor viruses is reviewed. The genome of the RNA tumor virus is described in terms of its physicochemical properties. Conditional (temperature sensitive, ts) as well as nonconditional mutants of type C viruses have been isolated. Many of the latter represent deletions. Three classes of mutants are identified according to their functional defects: class T mutants lack transforming ability; class R mutants cannot synthesize infectious progeny; and class C mutants have a defect in a viral gene required for transformation and replication alike. Examples of each are cited. Recombination is common among RNA tumor viruses and was first observed in double infections with nondefective avian sarcoma and avian leukosis virus having a different envelope specificity. All markers of RNA tumor viruses appear to be able to participate in genetic recombination. Recombinants must arise by molecular crossing-over between homologous nucleic acid molecules.

310. GENETICS OF AVIAN RNA TUMOR VIRUSES: A REVIEW OF RECENT DEVELOPMENTS.

Vogt PK, Linial M, Mason WS, Weiss RA, Wyke JA, Friis RR

Univ. Southern California Sch. Medicine, Los Angeles, CA

Biochem Dis; 4(PartB):619-630 1974

Recent results obtained in genetic studies of temperature-sensitive mutants of avian sarcoma viruses and with endogenous RNA tumor viruses are reviewed. There is now

ample genetic support for the conclusion that transformation by RNA tumor viruses requires the continuous activity of genes carried by the virus. However, genes carried by a virus are not necessarily viral genes. Viruses that become integrated in cellular DNA may also incorporate cellular genes, and these may be of importance in transformation. It is still too early to tell whether the two coordinate mutants reviewed here have a lesion on their DNA polymerase. If they do, they could answer many questions concerning the role and mode of action of this unusual enzyme. The discovery of genetic recombination between exogenous and endogenous RNA tumor viruses offers an explanation for some forms of apparent host-induced modification of RNA tumor viruses. Recombinants with endogenous virus can be selected for in certain host cells. For instance, a subgroup B virus stock containing a small fraction of endogenous subgroup E recombinant would become entirely subgroup E through selection if grown on cells that exclude subgroup B but admit subgroup E. Endogenous RNA tumor viruses are obviously subject to cellular genetic regulation, but the mechanisms of repression and of induction are obscure. Genetic variations among these viruses and their oncogenic potential also remain to be demonstrated.

311. AVIAN RNA TUMOR VIRUSES: MUTANTS, MARKERS, AND GENOTYPIC MIXING.

Vogt PK, Wyke JA, Weiss RA, Friis RR, Katz E, Linial M

Univ. Southern California Sch. Med., Los Angeles, CA

Collect Pap Annu Symp Fundam Cancer Res; 25:191-205

1974

Genetic studies on avian RNA tumor viruses are discussed with respect to conditional mutants, nonconditional mutants and genotype mixing. Conditional mutants include mutants which show a coordinate effect on transformation and virus synthesis, mutants which are temperature sensitive in virus replication but not in cellular transformation and mutants which are temperature-sensitive in transformation but not in virus replication. Nonconditional mutants, derived from helper-independent avian sarcoma viruses, are either transformation-defective or replication-defective. Genetic reassortment is found in several crosses between avian sarcoma and leukosis viruses. The conditional mutants show that viral genes are required for the initiation and maintenance of cellular neoplastic transformation. However, the spontaneous appearance of transformation-defective particles which are still able to produce infectious progeny indicates that at least some of the genes needed in transformation are nonessential for virus reproduction. It is suggested that some RNA tumor viruses might be transducing agents and that transduction of genes could be important in neoplastic transformation. Genetic events leading to the spontaneous appearance of defective and reassorted viruses probably reflect the structure of the viral genome. Evidence suggests that the 70S RNA of avian RNA tumor viruses is composed of subunits of about 35S which are linked by hydrogen bonds. The replication process may at one stage produce dissociated RNA subunits which are then assembled into progeny viruses. The transformation-defective and replication-defective particles may be among the viruses that receive less than a full complement of RNA subunits. Double infections would lead to mixing of subunits derived from different parents and could account for the high incidence of genetic combination from unmixed harvests. The segmentation of the genome places the RNA tumor viruses in a category with the reo and influenza viruses. The spontaneous emergence of defective particles is common but not unique to viruses with a segmented genome; defectives with deletions may also occur in viruses whose total genetic information resides in a single molecule.

312. AVIAN RNA TUMOR VIRUSES: A MODEL FOR STUDYING TUMOR ASSOCIATED CELL SURFACE ALTERATIONS.

Kurth R, Bauer H

Imp. Cancer Res. Fund Lab., London, England
Biochim Biophys Acta; 417(1):1-23 1975

A description of the avian tumor virus system, the biochemical detection of membrane changes in avian tumor virus-transformed cells, and immunological detection of membrane changes in those transformed cells is presented. Avian RNA-containing tumor viruses are C-type viruses, and are classified into avian sarcoma and avian leukosis viruses. The biological properties of individual strains are distinct with respect to the histological type of target cell. Studies attempting to establish consistent differences in the biochemical composition of malignant and corresponding normal cells measure the relative content and distribution of sialic acid, glycolipid phospholipids, glycoproteins, plus the differential interaction of cell surfaces with lectin. In vivo studies of tumor-specific transplantation antigens suggest a group-specificity for various avian sarcoma virus strains; contrasting experiments indicate transplantation immunity induced only by virus envelope antigen. In vitro studies of tumor antigens demonstrate the existence of two kinds of virus-directed cell surface antigens: the virus envelope antigens and the tumor-specific cell surface antigen system. Evidence is presented for the existence of common tumor antigens on avian tumor virus-transformed cells of different species, common tumor antigen on avian tumor virus-transformed sarcoma and leukemia cells, and for the reexpression of embryonic antigen paralleling transformation by avian sarcoma virus. The use of virus mutants in cell membrane studies indicates that continuous expression of certain viral genome products is linked with the transformed phenotype of the mutant-infected cells, and that synthesis of the gene product is inhibited under nonpermissive conditions. In investigating the immunological significance of new cell surface antigens, the demonstration of humoral and cellular immunity against tumor cells in the avian tumor virus system agrees with the general concept of 'immune surveillance'. Four possibilities are presented for the origin of tumor-specific cell surface antigen, plus the suggestion that it may represent a complex of several antigens including species-specific and interspecies-specific antigens. (135 refs)

313. THE CELLULAR AND MOLECULAR BIOLOGY OF RNA TUMOR VIRUSES, ESPECIALLY AVIAN LEUKOSIS-SARCOMA VIRUSES, AND THEIR RELATIVES.

Temin HM

McArdle Lab., Univ. Wisconsin, Madison, WI
Adv Cancer Res; 19:47-104 1974

RNA tumor viruses are medium-sized animal viruses with an RNA genome in the virion; virus replication involves a DNA intermediate and the virions contain a DNA polymerase. Rnadenaviruses include avian leukosis-sarcoma virus, mammalian C-type rnaadenavirus, viper C-type rnaadenavirus, mouse mammary tumor virus, Mason-Pfizer monkey virus, Visna virus, syncytium-forming virus, reticuloendotheliosis virus, and parties related to rnaadenaviruses. Virions of all rnaadenaviruses are medium-sized, roughly spherical and have a lipid-containing envelope with two external glycoproteins; inside the lipid envelope are 3-5 major proteins, a DNA polymerase, and RNA. Evidence for a DNA intermediate in the replication of Rous sarcoma virus (RSV), and for all RNA tumor viruses and rnaadenaviruses as proposed in the provirus hypothesis, is now quite strong. Sequences homologous to much of the RSV genome are present in DNA of RSV-infected cells and sequences homolog-

ous to a smaller fraction of the RSV genome are present in the DNA of uninfected chicken cells. It appears most likely that the DNA provirus is synthesized by the virion DNA polymerase with viral RNA as a template and primer; evidence includes the formation of provirus in the presence of cycloheximide, absence of DNA polymerase activity in certain noninfectious RSV and MSV virions, correlation of inactivation of infectivity and DNA polymerase activity after pretreatment of virions with inhibitors, and the existence of two temperature-sensitive RSV mutants with temperature-sensitive DNA polymerase activity. Most neoplasias are not associated with the presence of overt infection by a rnaadenavirus. The oncogene hypothesis states that the entire genome of a strongly transforming rnaadenavirus is present in all cells and that its activation leads to neoplasia. Hypothesis 3b of Temin suggests that RNA tumor viruses provide only an analogy for most neoplastic transformation; carcinogenesis involves the formation in cells of genes for neoplastic transformation by RNA-directed DNA synthesis.

314. TEMPERATURE-SENSITIVE MUTANTS OF AVIAN SARCOMA VIRUSES. (PP. 61-76)

Friis RR

The Biology of Tumor Viruses Proceedings of the Thirty-fourth Annual Biology Colloquium. Beaudreau G S, Snyder S, ed. Corvallis OR, Oregon State Univ Press, 1976.

Data from several laboratories, using a variety of techniques, to identify individual defective functions in temperature-sensitive mutant avian sarcoma viruses are summarized. Although these mutants can be categorized as either replication, transformation, or coordinate function mutants, nine different types are characterized functionally. To reduce possible effects of viral genetic background, chick embryo cells free of expression of viral functions were used. Viral functions were recognized by a temperature shift experiment in which cells are grown at permissive (35 C) and nonpermissive (41 C) temperatures; the complementation test, which involves establishing a mixed infection of two different viruses, mutants or wild-type, in the same cell; in vitro assays for reverse transcriptase activity; radioimmunoprecipitation tests for detection of group-specific (gs) antigens; and molecular hybridization to determine the production of new progeny viral RNA. Code no. 1 mutants, such as ts335 and ts337, are defective in reverse transcriptase. The mutant ts336 (code no. 2) is identical to code no. 1 mutants, except for apparently viable reverse transcriptase. Code no. 3 mutants, ts338 and ts343, are characterized by a reversible defect that affects both replication and transformation. Late transformation mutants, which are able to replicate progeny but are unable to transform the host cell at 41 C, were arranged into four distinct complementation groups and are designated by code nos. 4, 5, 6, and 7. At least one of these groups may be defective in a function that regulates some host cell genetic information. Transformation studies show a continuum of interacting alterations in cell behavior and biochemistry, each being determined by one or more viral functions. Code nos. 8 and 9 refer to late replication mutants that are blocked in the replication of infectious progeny. The analysis of functions that act on the host at the genetic level versus those that act on a specific cellular site may be of general significance in cancer research. (37 refs)

315. RAPID SCREENING ASSAY FOR REVERTANTS OF MURINE SARCOMA VIRUS-TRANSFORMED CELLS.

Nomura S

Lab. Viral Carcinogenesis, NCI, Bethesda, MD 20014
Methods Cell Biol; XIV:229-236 1976

A rapid screening assay for revertants of murine sarcoma virus (MSV)-transformed cells is presented. Reversion

frequency was tested in two sublines of 3197, 3-321 and 3-360, in the Ki-BALB line, and in S+L- revertants retransformed by MSV. All cell cultures were grown and maintained after virus infection in modified McCoy's 5a medium with 10% fetal calf serum. Cells were infected in suspension with murine leukemia virus (MuLV) at a multiplicity of infection (moi) of over five focus-inducing units (FIU) per cell, 1,000-5,000 cells in 5 ml of medium were delivered into 25-cm² flasks, and the flasks were incubated at 37 C. After 4 days, the flasks were shaken to detach the relatively loose cells, which were removed in the supernatant. Cultures were washed twice with medium and then refed with 5 ml of fresh medium. After a further 3-4 days of incubation, flat, contact-inhibited revertant cell colonies formed that were then counted. When S+L- cells were infected with MuLV at high moi (more than 5 FIU), MSV was rescued from them approx 17 hr after infection. At that time, rescue-negative revertant cells underwent MuLV infection, viral interference developed, and no transformation of revertants was possible by the rescued MSV. Revertants remained flat and formed colonies. The rapid screening assay allowed detection of revertant colonies in mixed populations containing a large excess of transformed cells. The procedure is not only useful for the determination of the spontaneous reversion frequency in MSV-transformed cells, but also for studying the reversion effects of physical, chemical, and biological agents that enhance the frequency of reversion. (13 Refs)

316. REVERSION IN CELLS TRANSFORMED BY TUMOR VIRUSES (MEETING ABSTRACT).

MacPherson IA

Imperial Cancer Res. Fund Lab., London, England
Proc R Soc Lond Biol; 177(1946):41-48 1971

The transformation of animal cells by DNA and RNA tumor viruses was discussed, with special reference to the phenomenon of 'reversion' of cell transformation, in which transformed cells lose their virus-transformed characteristics. In some cases of reversion, the transformed cells seem to lose the viral genes entirely; loss may occur spontaneously, as in the loss of the Rous sarcoma virus genome by transformed baby hamster kidney cells, or loss may be induced by chromosome loss in the transformed cells, as in the reversion of polyoma virus infection in transformed baby hamster kidney cells. In other instances of reversion, the viral genes remain in the transformed cells, but in an inactivated state, as in the conditional lethal mutants of polyoma virus and avian sarcoma virus. Reversion can be induced by culturing transformed cells on glutaraldehyde; polyoma virus-transformed hamster cells lose some of their transformed properties in these circumstances. Reversion may also be accomplished by killing transformed cells with fluorodeoxyuridine or bromodeoxyuridine and light. Transformed cells treated with these agents have been shown to lose some of the characteristics of virus-transformed cells, at least temporarily. (25 refs)

317. RNA-DEPENDENT DNA POLYMERASE IN VIRUSES AND CELLS: VIEWS ON THE CURRENT STATE.

Gallo RC

Natl. Cancer Inst., Bethesda, MD
Blood; 39(1):117-137 1972

The presence of an RNA-dependent DNA polymerase or reverse transcriptase has been found in every RNA oncogenic virus investigated, with the exception of some mutant viruses that have lost the ability to infect or transform normal cells. It is assumed that the role of the enzyme is to convert viral 70S RNA to a DNA copy allowing the viral genome to be inserted into host cell DNA. Nononcogenic viruses either contain no polymerase activity or an RNA-dependent RNA polymerase

with the exception of the Foamy viruses which have been shown to contain reverse transcriptase. However, it is thought that this type of virus may eventually be shown to be oncogenic. A demonstration of the presence of a true reverse transcriptase in an isolated particle would strongly support the notion that the particle is an RNA virus and potentially oncogenic. The presence of a true reverse transcriptase (catalyzing synthesis of DNA from pure single stranded natural RNAs) in cells is more difficult to establish. The use of some synthetic templates such as the RNA-DNA hybrids poly rA.dT to detect the presence of reverse transcriptase has led to some confusion. Although reverse transcriptase has great affinity for these templates they lack specificity, i.e., virtually any DNA polymerase will use them. Thus, activity with these templates does not in itself indicate that reverse transcriptase is present. At this time there is no published definitive information demonstrating the presence of this enzyme in any normal cell. An unequivocal demonstration of its presence in a human tumor tissue would, therefore, appear to indicate that information from an RNA oncogenic virus may be present.

318. TUMOR VIRUS GENETICS.

Kit S

Baylor Coll. Med., Houston, TX

Collect Pap Annu Symp Fundam Cancer Res; 25:143-157 1974

Studies with viral mutants can provide a direct way of relating viral gene functions to biochemical changes in infected cells. Several classes of oncogenic simian virus 40 (SV40) and polyoma virus mutants and of leukemia and sarcoma virus mutants are briefly reviewed. The SV40 and polyoma virus mutants are classified as: a plaque morphology mutants, deletion mutants, adenovirus-SV40 hybrids, defective lysogens, host-dependent mutants and temperature-sensitive mutants. Representative leukemia and sarcoma virus mutants (avian and murine) are: host range variants, sarcoma virus mutants which transform chick embryo iris epithelium cells to round, nonpigmented cells or to long fusiform cells, noninfectious C-type particles, a transforming virus strain that replicates only with the assistance of a helper leukemia virus, nontransforming strains isolated from helper-independent sarcoma virus, mutant sarcoma virus with transforming ability but no capability to replicate, and temperature-sensitive mutants. Properties of three defective murine sarcoma strains and one avian sarcoma strain are compared.

319. GENETICS OF DNA TUMOR VIRUSES.

Echart W

Salk Inst., San Diego, CA

Annu Rev Gene; 8:301-307 1974

The genetic properties of polyoma virus and simian virus (SV40), obtained largely by study of viral mutants and other mapping techniques are reviewed. It is suggested that knowledge of the genes of these transforming oncogenic viruses may help to pinpoint the mechanism of carcinogenesis, at least viral carcinogenesis. The review is presented under the following headings: infection and transformation by polyoma viruses and SV40, temperature-sensitive (ts) mutants of polyoma and SV40, host range mutants, deletion mutants, hybrid viruses, tsA mutants of polyoma and SV40, ts101 mutant of SV40, and late mutants of polyoma and SV40. The involvement of viral genes in transformation is discussed with particular evidence from ts viral transformation. The relationship of viruses to cell growth control is considered as only one of many factors in this complex process.

320. PROTEINS OF POLYOMA VIRUS AND SV40.

Crawford LV

Imperial Cancer Res. Fund. London, England
Br Med Bull; 29(3):252-258 1973

Several properties of polyoma virus and the closely related simian virus 40 (SV40), above all their small size, have made them popular model tumor viruses. The virus particles are about 45 nm in diameter and each particle contains a molecule of double-stranded DNA with a molecular wt of about 3.4×10^6 daltons, i.e., each contains 5500 base pairs. They are thus among the smallest DNA-containing viruses; only the parvoviruses, which contain single-stranded DNA, are smaller. The small size of the genome means that these viruses should be simple, both in structure and in their interaction with cells, because of their restricted coding potential. The basic virus proteins are histones, and the histones VP4, VP5, and VP6 are similar for the two viruses. These proteins are coded for by the host, and they occur only in DNA-containing particles. The remaining virus proteins, VP1 and VP2 (and VP3 for polyoma), seem likely to be virus coded in that they appear in cells only after infection. Proof of their being virus coded would be the *in vitro* synthesis of each protein under the direction of viral DNA of known purity. In both polyoma virus and SV40, there seem to be two groups of mutants which can complement each other, exert their effects late, and reduce the temperature stability of progeny virus particles. This is consistent with a particle's containing at least two virus-coded proteins. The small number of stably transformed DNA virus cells may be related to failure to complete late functions.

321. GENETICS OF ADENOVIRUSES.

Ginsberg HS, Young CS

Dept. Microbiology, Coll. Physicians and Surgeons, Columbia Univ., New York, NY
Adv Cancer Res; 23:91-130 1976

The mutagenesis and selection of adenovirus mutants, their use in genetic studies, their phenotypes, and their usefulness in experiments on the regulation of adenovirus replication and cellular transformation are reviewed. Although the adenovirus genome can potentially code for 50 or more proteins, unique mutations have been detected in only 10-12 genes. Of the number of conditionally lethal, temperature-sensitive (ts) mutants isolated, there is considerable clustering of two phenotypes, hexon and assembly mutants. The mutants isolated follow predictive intergenic reactions. The grouping of hexon mutants in a single cistron confirms chemical and physical evidence that the hexon is formed from the assembly of three identical polypeptide chains. Synthesis, assembly, and transport of hexon capsomers require more than a single gene product. The detection of three nonoverlapping complementation groups representing three genes essential for production of a functional fiber implies that the fiber consists of three rather than two unique species of polypeptides. Types 5 and 12 ts mutants have revealed a minimum of three viral proteins essential for initiation of DNA replication, but only one gene product has been identified so far, a DNA-binding protein that prefers single-stranded DNA. Experiments with two of these mutants, H5ts125 and H5ts149, have confirmed that late transcripts can be made only after replication of viral DNA has begun. The three mutants, H5ts125, H12ts307, and H12ts401, transform significantly more cells than wild-type viruses or other ts mutants. H5ts125 has been most thoroughly characterized and, as previously noted, is defective in a virus-specific, single-stranded DNA-binding protein. The protein of the viral genome that codes for the ts 125 gene product, which is approximately 0.65 physical map unit from the left-hand end of the genome, is rarely integrated in adenovirus-transformed cells. Three independently isolated mutants present this same

phenomenon of increased transforming capacity, whereas three other DNA-minus mutants, belonging to another complement group (H5ts3, and 37 and H5ts149), show either decreased or normal transformation frequency. It appears that the adenovirus DNA-binding protein plays a regulatory role in transformation as well as in DNA replication. (140 refs)

322. CELL TRANSFORMATION BY DNA-CONTAINING VIRUSES.

Rapp F, Westmoreland D

Dept. Microbiology, Milton S. Hershey Medical Center, Pennsylvania State Univ., Coll. Medicine, Hershey, PA 17033

Biochim Biophys Acta; 458(2):167-211 1976

A comprehensive review is presented of *in vitro* transformation by DNA viruses (papovaviruses, human adenoviruses, herpes simplex viruses, and other herpesviruses) and of the properties of the transformed cells, including the persistence of virus-specific functions. Although every cell in a culture transformed by RNA tumor viruses is capable of continuously producing and shedding mature infectious virus, this has not been observed in any cell line transformed by a DNA tumor virus. It is generally the case that a cell in which progeny virus is produced dies as a result of the infection. In addition, even when no progeny are produced, the expression of certain viral genes, such as those coding for virus structural proteins, may be incompatible with cell survival. Thus, expression of the transforming properties of DNA viruses requires that the virus-cell interaction be nonpermissive for the lytic cycle of the virus. This has been achieved by the following means: (1) the use of cell types that the virus can infect but in which replication is restricted; (2) the use of conditional lethal virus mutants under conditions not permissive for virus growth; (3) the use of virus preparations whose infectivity has been reduced, e.g., by UV irradiation; (4) experimental manipulation, such as growth in the presence of specific antiserum or infection using very low (or very high) input multiplicities that inhibit virus growth. (377 refs)

323. INTEGRATION OF DNA TUMOR VIRUS GENOMES.

Martin MA, Khoury G

Natl. Inst. Allergy and Infectious Diseases, Natl. Insts. Health, Bethesda, MD 20014

Curr Top Microbiol Immunol; 73:35-65 1976

Controlled hybridization analyses are the most direct approach for demonstrating the integration of viral DNA sequences within the host genome. Evidence for the continued presence of viral DNA in simian virus 40 (SV40) transformed cells was first obtained in virus-rescue experiments; data indicated that the induction mechanism requires fusion of the virus-free transformed cell with the permissive cell. Integrated viral genetic information can be detected by procedures utilizing separated strands of viral DNA, an analysis of DNA reassociation kinetics, or reconstruction and calibration experiments. Since infectious SV40 can be recovered from virus-free transformed cells by cocultivation or fusion with permissive African green monkey cells, the viral genome is probably present in a latent form. Findings from experiments on SV40 genome equivalents in transformed cells suggest that a single copy of SV40 DNA is sufficient to transform a cell. The presence of multiple copies of viral DNA in transformed cell lines cannot be correlated with any phenotypic difference or the amount of virus-specific RNA present. Although viral DNA can be detected in several virus-free transformed cells, the physical relationship of the viral genome to host cell DNA has not been fully resolved. Most reports concerning the relationship of papovavirus and adenovirus DNA's to cellular DNA suggest that viral DNA is integrated into the cell genome. Recent studies with temperature-sensitive mutants of

SV40 suggest that a viral gene product is necessary for continued maintenance of the transformed state.

324. TRANSFORMATION-DEFECTIVE MUTANTS OF POLYOMA VIRUS. (PP. 85-87)

Benjamin TL

Onco-Developmental Gene Expression. Papers Presented at a Conference Sponsored by the International Research Group for Carcinoembryonic Proteins, and Held at San Diego, May 29-June 1, 1976. International Research Group for Carcinoembryonic Proteins, San Diego, California, 1976.

The discovery of mutants of a viral gene that have a restricted host range (hr) and are unable to induce neoplastic transformation (-t) is reported. Nineteen of these hr-t mutants have been isolated from polyoma-transformed 3T3 cells. The mutants were derived from NG-18 and were unable to induce a cell surface alteration characteristic of transformed cells; this may underlie their inability to cause neoplastic transformation. These mutants were subsequently grown on C-type RNA virally infected 3T3 cells and on primary mouse embryo fibroblasts. Marker experiments indicated that a single wild type viral DNA restriction enzyme fragment can restore both a normal host range and the transformation ability of the mutants. Hr-t mutants, as well as particles of wild-type virus, contain histones derived from the cell. Histones H-3 and H-4, derived from the wild type, showed more extensive acetylation than normal host cell chromatin, while the same two histone fractions from mutant particles showed little increase in acetylation over the host. (11 Refs)

325. GENOME OF A SMALL TUMOR VIRUS. (PP. 111-157)

Nathans D

The Harvey Lectures Delivered under the Auspices of The Harvey Society of New York, 1974-1975. The New York Academy of Medicine, New York, Academic Press, Series 70, 1976.

The current status of research on simian virus 40 (SV40) is reviewed in a society lecture. The general properties of SV40, cleavage map of the SV40 chromosome, mapping of SV40 template functions, including initiation, termination, and transcription sites for DNA replication, gene mapping, temperature sensitive mutation, evolutionary variation, generation of deletion mutants after enzymatic modification of SV40, and activity of SV40 deletion mutants are topically reviewed. Restriction endonucleases have shown the SV40 chromosome as a covalently closed circular molecule of duplicate DNA with approximately 5300 nucleotide pairs. The single site of initiation of bidirectional DNA replication has been established at 0.67 map units. The initiation signal is the only cis-element required for replication. Half of the molecule is the template for early viral RNA and most of the remaining RNA in transformed cells; the other half of the molecule is the template for late RNA. Early and late RNA are transcribed counterclockwise and clockwise, respectively. The proximity of the unique initiation site of DNA replication to the 5'-ends of early and late SV40 messenger RNA suggests the structural relation between initiation of viral DNA replication and transcription. (92 refs)

326. DEATH AND TRANSFORMATION. (PP. 287-300)

Martin RG, Anderson JL

Biology of Radiation Carcinogenesis. Edited by Y. M. Yuhas, R. W. Tennant, J. D. Regan. New York, Raven Press, 1976

The role of SV40 in inducing transformation in tissue cultures of the Chinese hamster lung (CHL) was examined. CHL cells were infected with A, B, C, BC, or D mutants of SV40 and grown on 2.5% serum. All transformants behaved

like control transformed cells at the permissive temperature and differently from normal, uninfected cells, but the A mutants (A207 and A239) acted like normal cells at nonpermissive temperature, indicating that the A function is required for rapid growth in low serum concentrations. T antigen (assayed by indirect immunofluorescence) is not contained in normal cells, but all mutants (A207, A209, A239, A241, B201, BC245, C219, and D202) exhibited T antigen at permissive (33°C) and nonpermissive (40°C) temperatures. The uptake of 2-deoxy glucose is not related to the other manifestations of SV40-transformed CHL and mouse brain cells. In relatively poor medium (Ham's F-12) at 33°C, wild-type and A239 transformed cells grow more rapidly than normal CHL cells and they grow beyond a cell density of 10^7 cells/cm². Neither fully transformed growth at 33°C nor temperature sensitive "revertant" behavior at 40°C depends on accompanying changes in chromosome number. A mechanism for SV40 transformation was proposed in which the degree of transformation can be explained by position effects on the expression of the SV40 genome; e.g., cells will be minimally transformed when SV40 is integrated in chromosome regions normally "turned off" and cells will be fully transformed when SV40 is integrated in chromosome regions expressed constitutively.

327. GENETIC TRANSFORMATION OF ANIMAL CELLS WITH VIRAL DNA OF RNA TUMOR VIRUSES.

Hill M, Hillova J

Dept. Cellular and Molecular Biology, Equipe de Recherche No. 148 du C.N.R.S., Inst. Cancerology and Immunogenetics, Villejuif, France
Adv Cancer Res; 23:237-297 1976

Experiments showing that animal cells become genetically transformed in vitro following infection with RNA tumor viruses are discussed. Infectious forms of the type C RNA tumor viruses carry an RNA-directed DNA polymerase, which, early in infection, transcribes viral RNA into DNA, the latter becoming covalently bound to the host cell chromosome. Transfection has shown that there is a DNA intermediate in the life cycle of the RNA tumor viruses that is integrated into the cellular genome. It is not clear whether the RNA-DNA and double-stranded DNA forms isolated in early infections are all precursors of the integrated viral DNA. It is possible that virus-infected cells contain, besides full-length viral DNA, partial transcripts that may escape detection in transfection assays. The integration mechanism inserting viral DNA into the cellular chromosome also remains unknown. The ability of both the sarcoma and leukemia viruses to cause malignant transformation depends on the presence of new genetic material, introduced by the virus into the infected cell, which can be detected by both nucleic acid hybridization and transfection. The viral genetic material of sarcoma viruses contains transforming genes that must be continually expressed to maintain the transformed phenotype of virus-infected cells. These transforming cells are not involved in virus replication. It is probable that these genes were originally mutated cell genes whose covalent linkage to viral nucleic acid sequences was favored by strong selection and passage in the laboratory. No temperature-sensitive (ts) leukemia virus mutants have yet been found that pinpoint transforming genes. Leukemia viruses almost certainly lack transforming genes found in sarcoma viruses, and it is possible that the genes responsible for at least certain leukemias are also involved in virus replication. It is not clear whether endogenous viruses represent a natural reservoir for accidental evolution of exogenous viruses, or whether endogenous and exogenous viruses diverge early in evolution. Some oncogenic type C viruses lack genetic relatedness to their natural hosts, and no endogenous counterparts of these viruses have so far been identified. It is possible that certain animal tumors (including

human leukemias) are due to products coded for by transforming genes of exogenous RNA tumor viruses. (305 refs)

328. CONTRIBUTIONS OF VIROLOGY TO ONCOLOGY.

Stoker MG

Imperial Cancer Res. Fund Lab., Lincoln's Inn Field,
London, England

Neoplasma; 22(3):243-249 1975

Contributions of virology to oncology are reviewed. Tumor viruses are studied for two main reasons: as agents of natural cancer, and as tools in general oncology. Viruses have proved to be invaluable for investigating the physiological changes in cancer cells. The study of virus transformed cells and untransformed controls is readily accomplished and the process can be studied by the use of revertants. Tumor viruses contain one or more 'transformation genes' which are probably analogous to cancer genes. Correlations have been established between viruses and naturally occurring cancers (as opposed to those experimentally induced). Among these 'cancer-inducing' viruses, polyoma virus, Simian virus 40 and Herpes virus are well known. Marek's disease (Herpes) induces lymphoma in chickens. Feline leucosis syndrome is also a viral cancer. Except in the case of benign papillomas, there is no clear proof of a viral cause of any human cancer. Burkitt's lymphoma, nasopharyngeal cancer and infectious mononucleosis all have the same virus present. Carcinoma of the cervix is paralleled with a positive Herpes II test. There is no conclusive epidemiological or genetic evidence of viral transmission of leukemia and mammary cancer in man, though B and C type particles are found in human milk and have been reported in leukemic tissue. It appears that oncogenes and/or virogenes are present in all cells and their activation by carcinogens is the general cause of cancer. (no refs)

329. THE CELL SURFACE AND MALIGNANT TRANSFORMATION.

Pollack RE, Hough PV

Cold Spring Harbor Lab., NY

Annu Rev Med; 25:431-446 1974

Properties of cells transformed by DNA or RNA tumor viruses have been described in detail, but mechanisms for maintenance of the transformed state remain unknown. New lines of research, much of it involving experiments on the surface of normal and malignant cells, are beginning to suggest possible mechanisms. A synthesis of results obtained at different laboratories working on widely different biologic systems is presented. Membrane proteins and glycoproteins, cyclic nucleotides, and growth stimulating factors are

examined in relation to cell transformation and proliferation. Special attention is given to two missing links in the understanding of control of proliferation: (I) the sequence of biochemical events occurring during stimulation of the surface and initiation of DNA replication and (II) the sequence of biochemical events leading from the integration of the genome of the oncogenic virus into the host chromosome to modification of the surface, or to modification of the proliferation control network (I). Changes in growth, surface, and other cell parameters are discussed and two useful virus cell systems are briefly considered (SV40-3T3 cells and temperature-sensitive mutants of avian sarcoma virus-chick fibroblasts). Two cellular effects close to the transforming virus are identified. One effect is the freeing of some surface receptors that are immobilized in untransformed cells; the second is the induction of a proteolytic activity which probably acts at the cell surface and may have the effect of freeing the receptors.

330. SURFACE CHANGES IN TRANSFORMED CELLS DETECTED BY LECTINS.

Burger MM

Dept. Biochem. Sci., Princeton U., NJ

Fed Proc; 32(1):91-101 1973

The use of plant agglutinins in assessing surface differences in transformed cells is reviewed including discussion of such topics as available lectins, lectin receptor sites and their respective topography, a quantitative assay for cell agglutination, the possible significance of surface alterations detected with lectins, and the possible consequences of these surface alterations for growth control. Increased agglutinabilities with lectins have been observed for many transformed tissue cultures as well as native tumor cells when compared with their normal parent cells. Only some lectins, however, are able to distinguish between transformed and untransformed cell surfaces and even these lectins are not able to detect differences between all pairs of normal and transformed cells. The positive correlation between increased lectin agglutinability and several aspects of transformation may be summarized as follows: 1) some 40 to 60 lines of transformed cells have been found to agglutinate well with various lectins; 2) tumor cells that were selected for reversion to good density-dependent inhibition of growth lose their agglutinability; 3) tumor cells that were selected for a loss of agglutinability regain to some extent their normal growth control; 4) conditionally nontransforming viral mutants and temperature sensitive mutants cannot achieve the agglutinable state under nonpermissive conditions, and 5) temperature-sensitive host mutants that lose some of their transformed properties at the nonpermissive temperatures lose their agglutinability simultaneously.

SUBJECT INDEX

- A protein, 75, 114
 Actin, 107, 260-264, 282, 304
 Adenoviruses
 Type 5, 25, 42, 43, 81, 94, 188, 321-323
 Type 7, 28, 173, 208, 317
 Type 12, 23, 26, 27, 217, 286, 321-323
 Type 31, 24
 Adenylate cyclase, 11, 270, 271, 273, 275
 Adhesion properties, 232-238, 245, 266
 Amino transferase, 297
 Anchorage, 87, 261, 281
 Aneuploidy, 139
 Anti-inflammatory Agents, 10, 75, 78
 Autolysis, 246
 Bromodeoxyuridine (BrdU), 9, 27, 30, 66, 101, 118, 124, 141, 145, 159-161, 163, 179, 189, 193, 194, 209, 316
 Cancer-prone Personality (see also Psychological Factors in Carcinogenesis)
 life-style, 19, 24, 31, 34, 36, 45, 54
 personality, 3, 28-30, 37-51, 54
 psyche, 39, 50, 63, 64
 psychosomatic factors, 32, 33, 41, 42, 44, 49, 50, 54
 cDNA-sarc, 53-55, 181
 Cells and cell lines
 3T3 cells, 9, 16, 20-22, 64, 67, 70, 73, 83, 84, 86, 87, 90, 104, 105, 108, 109, 112, 113, 142-154, 156-158, 161, 162, 166-169, 178, 182, 186, 187, 190, 191, 194, 196-199, 205, 207, 218, 228, 231-236, 239, 247, 248, 254, 255, 260-263, 266, 268, 272, 277, 281, 324, 329
 African green monkey cells, 16, 68, 69, 74, 79, 81, 92, 173, 196, 323
 Chuck embryo, 1, 3, 5, 7, 8, 11, 33, 42, 45, 90, 54, 61, 101, 102, 116-126, 179, 215, 216, 220-223, 225-227, 230, 251, 237, 238, 240-242, 245, 246, 249, 251, 256-259, 264, 267, 269-271, 273, 275, 276, 280, 284, 285, 288, 289, 292, 294-300, 301, 302, 304, 305, 307, 314, 318
 Hamster embryo, 12, 25, 28-30, 94, 108, 109, 129, 131, 135, 137, 140, 141, 185, 212, 243, 285
 Hamster kidney, 14, 20, 21, 23, 60, 66, 82, 94, 108, 109, 129, 130-133, 136, 139, 141, 175, 179, 181, 192-194, 200, 201, 204, 229, 244, 278, 285, 287, 293, 297, 316
 Hamster lung cells, 40, 41, 72, 106, 174, 279, 326
 L cells, 44, 196, 209-211, 214
 NRK cells, 39, 173, 237, 214, 285
 Rat cells, 4, 43, 74, 76, 83, 86, 92, 122, 154, 187, 202, 214, 252, 265, 274, 282, 291, 297
 SV 3T3 cells, 142, 144, 161, 167, 247, 263, 266, 279
 Endomet, 187
 Colonies, 144, 216
 Complementation, 11, 14, 18, 36, 37, 29, 32, 35, 40, 41, 43, 56, 68, 75, 87, 92-94, 110, 120, 173, 212, 213, 250, 306, 314, 321
 Immunization & 36, 105, 113, 134, 136, 132, 152, 153, 157, 158, 162, 166, 168, 173, 196, 213-220, 233-235, 247, 260, 263, 265, 285, 309
 Genetic inheritance, 16, 106, 112, 113, 130, 133, 136, 344, 148, 180-181, 184, 185, 187, 232, 233, 237, 243, 235, 268, 317
 Fertilization, 97
 Leucine AMP, 236, 10, 109, 232, 268, 269, 270, 273-276
 Cyclic GMP, 272, 274
 Deletion map, 57
 Experimental Tumorigenesis
 burns, 82
 cage conditions, 69-73, 103, 116, 118, 119, 123
 cold, 96, 99, 105, 106
 crowding, 94, 110, 125
 electric shock, 71, 89, 90, 92, 94, 95, 103, 105, 111, 125
 environmental stress, 97-100
 exercise, 91, 100, 125
 handling, 69, 70, 72, 95, 105, 118, 125
 light, 101, 111
 meteorological stress, 124
 motion, 102
 nutritional stress, 74, 77, 115
 radiation, 96-98, 107, 108
 restraint, 67, 68, 88-91, 103, 104, 109, 117, 122
 sound stress, 90, 93, 94, 120-122
 surgical stress, 74, 107, 108, 115
 starvation, 87, 114
 transportation, 105
 viruses, 81, 82, 84, 85, 112, 113, 116-125
 Flat variant, 157, 158
 Fluorodeoxyuridine, 71, 85, 157, 162, 169, 175, 176, 248, 316
 GAG gene, 64, 247
 Galactoprotein, 244
 Gene A, 69, 75, 107-109, 111, 250
 Glycolipid, 240, 244, 250, 302, 312
 Glycoproteins, 8, 10, 38, 51, 52, 65, 122, 124, 221, 224, 228, 232, 233, 237, 240, 242, 243, 245, 250, 266, 291, 302, 304, 306, 312, 313, 329
 Glycosidase, 246
 Helper viruses, 1, 2, 4, 8, 33, 34, 46, 47, 58, 83, 93, 100, 123, 151, 164, 190, 306, 311, 318
 Herpes viruses
 Herpes simplex type 1, 29, 30, 31, 32, 44, 60, 189, 209, 210, 211, 214, 322
 Herpes simplex type 2, 28, 29, 30, 31, 60, 209-214, 321, 328
 Other herpes, 221, 322, 328
 Heterokaryon, 16, 180, 196
 Hexose uptake, 109, 119
 Histones, 76, 89, 120, 324
 Host range properties, 2, 6, 11-34, 37, 59, 65, 83, 84, 86, 87, 89, 94, 116, 177, 190, 191, 318, 319, 324
 Hyperplasia, 142
 Immunological Factors
 host resistance, 116
 immunity, 4, 28, 58, 59, 83, 77, 81, 105, 107, 108, 112, 115, 118, 119
 immunoglobulins, 57, 58, 71
 immunological factors, 29, 31, 32, 34, 66-72, 76-79
 T-cells, 55, 58, 69, 70, 72, 113, 116
 thymus, 55, 69-72, 76, 79, 80, 84, 85, 112, 113, 116, 117, 119, 122
 spleen, 53, 69
 lymph nodes, 53, 69
 Interferon, virus, 40, 86, 93, 123, 179, 183, 185, 186, 188, 194, 201-204, 207, 321-323, 326, 327, 329
 Isopropyl, amino transferase, 297
 Leucine, 87, 88, 113, 216, 220, 312, 319

- Leukemia viruses**
 Avian, 53, 41, 318, 327
 Murine, 56, 57, 62-64, 87, 100, 111, 148, 154, 156-158, 161, 162, 164, 178, 182-184, 190, 191, 205, 232, 274, 282, 291, 315, 318, 327
- Leukosis virus**, 1, 4, 8, 10, 33, 37, 46, 47, 57, 99, 125-128, 241, 245, 246, 289, 306, 309, 311-313
- Lipid**, 240, 248, 249, 302, 304, 313
- Microfilaments**, 238, 260, 263, 264, 266, 267, 282
- Microtubules**, 151, 238, 263, 267, 282
- Myoblast**, 299
- Neoplasms**
 cervical, 1, 6-8, 15-18, 22, 23, 34, 35, 39, 41
 endometrial, 17, 41
 epidermoid, 106
 gastric, 33, 46, 104
 gastrointestinal, 58
 hepatic, 101, 102
 Hodgkin's disease, 9, 11
 leukemia, 8, 9, 11, 32, 35, 43, 94
 lymphoma, 8, 53, 58, 114
 lymphosarcoma, 113
 mammary, 1-4, 14, 21, 28, 30, 35, 37-39, 41, 46, 52, 56, 57, 61, 62, 103, 105, 116, 118, 119
 melanoma, 110, 113
 ovarian, 35, 41
 pancreatic, 11, 19, 20, 25
 pulmonary, 11-13, 35, 40, 46, 48, 86
 sarcoma, 5, 123
 other, 1, 41, 106, 120
- Neuroendocrinological Factors**
 adrenal gland, 55, 68, 80, 84, 85, 88, 90, 91, 96, 102, 103, 111, 114, 117, 121, 123, 124
 adrenaline, 95, 107-109, 121
 autonomic system, 33, 63
 corticosteroids, 2, 55, 56, 62, 66, 68-70, 72-76, 79, 85, 93, 103, 108, 110, 112, 113, 116, 119, 122
 cortisol, 59, 61, 107, 108
 glucocorticoids, 80, 82, 86, 107
 hormonal factors, 4, 25, 29, 42, 54, 66-76, 78, 79, 111
 hypothalamus, 59, 63, 67, 71
 nervous system, 31, 32
 neuroendocrine system, 55, 63, 64, 90, 92, 103
 pituitary, 59, 65, 67, 68, 89, 102
 steroid hormones, 5, 16, 56, 62
- 4-Nitroquinoline-1-oxide**, 175
- Nitrosomethylurea**, 175
- Nonproducer cells**, 39, 56, 57, 63, 123, 154, 158-162, 164, 173, 190, 191
- Oligomer**, 197-199, 280
- Plasmid**, 97
- Pol(A)**, 49
- Polyamine**, 295, 296
- Polyoma virus**, 12-15, 20-22, 58, 59, 66, 67, 70, 71, 73, 77, 80, 82, 84-89, 104, 105, 112-114, 129-141, 149, 163, 192, 193, 197-202, 215, 229, 239, 244, 248, 254, 277, 278, 297, 316, 318-320, 324, 328
- Pregnancy**, 4, 7, 67, 73, 113
- Proteases**, 124, 221, 226, 246, 257, 304
- Psychological Factors in Carcinogenesis (See also Cancer-prone Personality)**
 affective disorders, 27, 29-36
 alienation, 29, 32
 anger, 37, 57
 anxiety, 8, 29, 35, 111
 behavior, 23, 24, 32, 54
 depression, 2, 8, 11, 27, 29-31, 35-37, 41, 46, 54
 despair, 32-34, 41, 44, 54
 ego defenses, 55, 56
 emotional stability, 6, 35
 emotional stress, 2-13, 29, 33, 35, 37, 39, 44, 46, 55, 58, 61, 62, 71, 75
 emotional expression, 37, 40, 43, 51, 57
 extroversion, 40
 hostility, 38
 isolation, 32
 life-event stress, 1, 8, 22, 34, 41, 45
 loss, 22, 29-33, 41, 42, 44
 mental illness, 39
 neuroticism, 8, 27, 30, 40
 psychological factors, 9, 31
 self-hatred, 42
- PTD mutant**, 124
- Recombination, genetic**, 8, 10, 11, 26, 29, 34, 35, 37, 38, 42, 43, 52, 56, 57, 99, 101, 103, 106, 309-311
- Replicon**, 40, 68, 201
- Rescue virus**, 39, 83, 90, 93, 123, 150, 154, 156-158, 160, 162, 164, 166, 168, 169, 173, 179, 180, 182, 186, 187, 190, 195, 200, 202, 205, 315, 323
- Retransformation**, 141, 146, 157, 168, 178, 192, 315
- Reverse transcriptase**, 62, 96, 100, 182, 313, 317, 327
- Reverse transcriptase deficiencies**, 38, 61, 157, 158, 164, 314
- Revertant cells**, 4, 8, 86, 87, 98, 99, 123, 125, 126, 130-140, 142-158, 160, 162-165, 175-181, 183, 189, 191-194, 205, 206, 218, 232, 235, 237, 239, 243, 247, 248, 251, 255, 260-262, 268, 272, 281-283, 289, 291, 293, 315, 316, 326, 328
- Risk Factors Combining with Stress**
 alcohol, 10, 16, 26, 28, 36, 54, 65, 75
 childhood, 32, 34, 40, 43-47
 diet, 4, 25, 28, 36, 61
 divorce, 14, 15, 17-22
 education, 6, 43
 genetic factors, 4, 10-13, 18-21, 46, 53, 87
 poverty, 18
 psychosocial factors, 1, 9, 43, 45-47, 63
 religion, 16, 20, 21
 sexuality, 6, 16, 20, 22, 23, 25, 28, 34, 39
 sociocultural factors, 20, 24
 socioeconomic level, 4, 10, 15, 20, 22, 30, 38
 widowhood, 14-16, 18, 21, 44
 tobacco, 10, 12, 13, 16, 28, 36, 40, 54
- Rorschach Test**, 9, 43, 48-50
- Sarcoma viruses**
 B77 strain, 3, 4, 11, 45, 46, 62, 65, 171, 225, 273, 275
 Bryan, 8, 34, 36, 46, 50, 95, 96, 101, 118, 119, 220, 227, 259, 269-271, 285, 301, 302, 305, 306, 307
 Murine, 39, 63, 100, 121-123, 146-148, 154-161, 164, 168, 178, 190, 191, 205, 206, 252, 262, 266, 274, 281, 282, 313, 315
 Rous, 1, 2, 4, 5, 7, 8, 10, 33-38, 46-52, 61, 62, 95-103, 115-120, 124, 127, 128, 154, 155, 163, 179, 180, 215, 219-231, 238, 240, 242, 245, 246, 249, 251, 253, 256-259, 261-271, 273, 275, 276, 280, 284, 285, 287, 288, 292, 294-308, 313, 316
 Schmidt-Ruppin, 5, 8, 11, 36, 38, 46, 50, 53, 115, 117, 154, 155, 163, 215, 216, 222, 223, 225, 229, 245, 246, 251, 263, 271, 273, 280, 285, 287, 294, 296, 299, 301, 304, 306, 307
- Screening Program**, 45-54
- Segregation, genetic**, 8, 33-35, 46, 47, 141, 178, 184, 193
- Sinan virus**, 40, 16-19, 28, 29, 40, 43, 68, 69, 72, 76, 78-81, 87, 91-93, 107-111, 143-153, 165-169, 173, 176, 177, 184-187, 194-196, 203, 204, 207, 208, 212, 215, 218, 228, 233-235, 239, 254, 255, 260-263, 272, 281, 293, 318-320, 323, 325, 326, 329
- Stress-related Diseases**
 autoimmune arthritis, 71, 77
 cardiovascular, 8, 66, 69
 coronary, 1, 5, 29
 psychosomatic, 26, 28

- schizophrenia, 26, 28, 30, 71
- ulcer, 10, 104
- ulcerative colitis, 58, 75
- Sugar transport, 153, 251, 253, 255, 258
- T Antigen, 16, 24, 27, 70-74, 77, 79, 83, 90, 93, 107, 108, 111, 132, 137, 141, 143, 149, 150, 152, 165, 166, 168, 169, 173, 177, 185, 194, 196, 207, 326
- Thymidine kinase, 17, 24, 26, 44, 66, 67, 189, 209-211, 214, 293
- Transcription, 57, 69, 79, 122, 123, 176, 179, 189, 207, 208, 298, 301, 325, 327
- Transplantation antigens, 79, 80, 133, 192, 312
- Type C viruses, 3, 10, 30, 39, 56, 57, 64, 87, 96, 117, 123, 156, 159, 164, 182-184, 205, 291, 309, 312, 313, 318, 324, 327, 328
- Vacuolization, 302, 305
- Viral Factors, 4, 15, 25, 53, 55, 116-122
- Vitamins
 - A, 12, 70, 80-86
 - E, 87
- Wheat germ agglutinin, 85, 105, 165, 168, 215
- Xenotropic virus, 191

BLANK PAGE

AUTHOR INDEX WITH CITATION NUMBERS

- Aaronson S.....121
 Aaronson SA9, 64, 100,
 123, 154, 155, 190
 Albert SO.....247
 Aldrich C.....23
 Altenburg BC.....282
 Ambros VR.....230
 Anderson GR.....123
 Anderson JL 80, 111, 326
 Anderson WB.....270, 271
 Anisowicz A.....57
 Anthony DD.....69
 Aoi Y.....250
 Ash JF.....265
 August JT.....294
 Aupoix MC.....287
 Austin PE.....43
 Avila J.....40
 Bacalao J.....291
 Bachelier LT.....199
 Bachrach U.....295, 296
 Bader AV.....302
 Bader J.....269
 Bader JP118, 119, 227, 259,
 301, 302, 305
 Balduzzi PC.....101
 Baltimore D.....96, 98, 99
 Bancuk JE.....201
 Barbacid M.....64
 Barlati S.....284, 285
 Basilico C20, 66, 67, 166,
 167, 169, 186, 202,
 207, 278, 303
 Bassin RH.....156, 205
 Bauer H.....124, 127,
 290, 312
 Baygell P.....72
 Beaman JA.....35
 Becker D.....124
 Beemon K.....52
 Bell JG.....35
 Benjamin T.....59, 88
 Benjamin TL84, 85, 86, 87,
 89, 324
 Benyesh-Melnick.....29, 212
 Benyeshi-Melnic.....63
 Berg P.....177, 198
 Bernstein A.....103
 Bershadsky AD.....283
 Beug H.....172, 267
 Biehl J.....360
 Biggs PM.....127
 Billeter MA.....49
 Biquard JM120, 216, 229,
 287
 Bishop JM 53, 54, 55, 181
 Black PH150, 151, 152, 247,
 260, 293
 Block-Shtacher.....139
 Blout ER.....248
 Blomberg PM.....304
 Bo-Chen L.....230
 Boettiger D.....167, 179, 180
 Boice L.....199
 Bolden A.....61
 Bosmann HB.....245, 246
 Bouck N.....175
 Bradley WE.....255
 Brady R.....239
 Brady TM.....257
 Branton PE.....223, 304
 Brockman WW.....93
 Brown NR.....118, 259, 301
 Brugge JS.....109, 173
 Buchanan JM.....230
 Buck CA.....242
 Buckman T.....257
 Buniel JF.....233, 234
 Burger MM85, 105, 215,
 330
 Burmester BR.....128
 Burstin SJ.....278
 Butel JS.....109, 173
 Buityan R.....211
 Bye JM.....221
 Cachard A.....287
 Callender J.....13
 Calothy G.....102
 Campbell G.....185
 Case KR.....245
 Chang C.....80
 Cho HY.....164
 Chou JY.....40, 41, 106
 Christensen M.....274
 Clements JB.....60
 Collins JK.....75
 Courtney RJ.....29
 Cowan K.....69
 Crawford LV.....320
 Critchley D.....124, 240
 Crittenden LB.....128
 Culp LA150, 151, 152, 153,
 232, 233, 234, 235,
 255, 260
 Cumar FA.....239
 Cutchins EC.....164
 Cuzin F.....73, 198
 Davis D.....209
 Defendi V.....185
 Delotto R.....297
 Deng CT.....179, 181
 DePamphilis M.....177
 Di Mayorca G.....13
 Di Mayorca G.....175
 Dieckmann M.....198
 DiMayorca G.....20
 Don S.....295, 296
 Dreesman GR.....63
 Drost S.....99
 Drost SD.....98
 Duhbs DR.....17, 187, 195
 Duesberg P.....48
 Duesberg PH36, 45, 46, 50,
 51, 52
 Dulbecco R18, 22, 77, 91,
 104, 105, 277
 Dunn KJ.....157, 158, 178
 Echart W.....319
 Eckhardt W.....104
 Eckhart W14, 21, 70, 71,
 105, 254
 Edelman GM.....263
 Eger RR.....231
 Ensinger M.....81
 Ensinger MJ.....188
 Esparza J.....29, 212
 Feunteun J.....59
 Fischinger PJ156, 157, 158,
 178, 191, 205, 206
 Fizman IY.....299
 Flannery VL.....30
 Fluck M.....59
 Folk WR.....200, 201
 Francke B.....70
 Frankel AE.....206
 Freedman VH.....281
 Frenkel G.....81
 Fried M.....15, 58, 82
 Friis R.....124
 Friis RR4, 6, 7, 171, 290,
 310, 311, 314
 Fuchs P.....248, 299, 304
 Fuhrer JP.....242
 Fujinaga K.....208
 Gaffney BJ.....304
 Gahmberg CG.....244
 Gallo RC.....317
 Gazdar A.....184
 Gelb NA.....247
 Gelfand IM.....283
 Gelfand VI.....283
 Gerwin BI.....157
 Gilden R.....159, 160
 Gilden RV.....161
 Gilula NB.....231
 Ginsberg HS.....27, 188, 321
 Giordano R.....13
 Glick MC.....243
 Goldberg AR.....264
 Goldberg RJ.....56
 Golde A.....5, 307
 Goldman E.....86, 87
 Goldman RD.....262
 Graf T.....127, 171, 172, 267
 Green H.....67
 Greenberger JS.....123
 Griffin BE.....58
 Grimes WJ.....150
 Groudine M.....298
 Guelstein V.....283
 Guntaka RV.....53
 Haapala DK 156, 205, 206
 Hackett AJ.....182
 Hakomori SI.....244
 Hakura A.....112, 113
 Hale AH238, 249, 256, 257
 Hamada C.....265
 Hamafusa H1, 2, 8, 38, 51,
 52, 51, 95, 96, 117,
 128, 306
 Hanafusa T.....51, 95, 306
 Hartley JW.....158
 Hatanaka M 159, 160, 161
 Hayashi M.....140
 Hayman MJ.....10
 Hill M.....127
 Hiller AJ.....218
 Hilova J.....327
 Hirai K.....185
 Hirschberg CB.....304
 Hitotsumachi S.....138
 Holtzer H.....300
 Horecker BL.....294
 Hough PV.....329
 Howard T.....39
 Howk RS.....57, 183
 Huebner RJ.....164
 Hughes RG.....44
 Humphries KC.....221
 Hunter E.....7, 10, 37, 62
 Hynes RO.....221, 226
 Ihara S.....11
 Ikawa Y.....275
 Inbar M.....132
 Irwin JC.....224
 Isa T.....225, 275
 Iwabashi M.....94
 Itagaki A.....110
 Ito Y.....77
 Iverson GM.....184
 Jainchill JL.....148
 Johnson GS.....269, 270
 Johnson M.....257
 Kaji A.....297, 300
 Kakunaga T.....174
 Kasten G.....204
 Katz E.....311
 Kauffman RS.....188
 Kawai S8, 38, 50, 51, 52,
 117, 306
 Khoury G.....323
 Kiehn D.....244
 Kimura G.....18, 91, 110
 Kimura S.....29, 30, 212
 Kingsbury EW.....218
 Kit S17, 63, 127, 127, 125,
 252, 318
 Klein R.....159, 160
 Kletzien R.....241
 Kletzien RF.....253, 258
 Komet RW.....31
 Kozai Y.....126
 Kraiselburd E.....189, 209
 Kram R.....272
 Kryceve C.....284, 285
 Kuchino T.....74, 92, 250
 Kurth R124, 125, 289, 290,
 312
 Kuwata T.....219
 La Rossa DD.....257
 Lazarides L.....262
 Ledinko N.....26
 Leiden JM.....211, 214
 Levine AJ.....81, 208
 Lev B.....30
 Lew MA.....259
 Lin SS.....210
 Linder E.....288
 Linial M.....97, 310, 311
 Littlefield JW.....66
 Livingston DM.....39, 72
 Lockwood MP.....203, 204
 Lockwood T.....246
 Loma CW.....160

Losasso L	238	Park WP	56	Schultz AR	153	Van Eldik LJ	276
Lovelace E	271	Parks WP	57	Schwartz M	75	Vanaman TC	276
Lund E	58	Parola A	248	Scolnick EM	56, 121, 154	Varmus HE	53, 54, 55, 179, 181
Lundholm U	188	Pastan	1236, 237, 266, 269, 270, 271	Scolnick EN	57	Vasiliev JM	283
Macnab JC	60, 213	Paulin D	73	Scott WA	93	Venuta S	251
Macpherson I	194	Pearlstein ES	221	Sekikawa K	208	Verma IM	62, 98, 99, 100
MacCormick R	103	Peebles PT	156, 183, 205	Shani M	137	Via DP	282
MacDonnell D	47	Perdue JF	241, 253	Sharp PA	176	Vigier P120	216, 229, 284, 285, 308
MacPherson I179	192, 240	Perez-Bercoff R	49	Sheinin R	228	Vogel A142	143, 144, 145, 146, 147, 162, 262
MacPherson IA	316	Pessac B	102	Sheppard JR	268	Vogt M	197, 198, 199
Mann J	209	Peters Jrl	267	Shih M	161	Vogt PK3	4, 6, 10, 33, 34, 37, 45, 46, 50, 54, 116, 128, 265, 309, 310, 311
Mapstone TB	235	Pollack P142	143, 144, 145, 146, 147, 261, 262, 281	Shimojo H	24, 208	Vokaer A	272
Marciani DJ	220, 227, 305	Pollack RE	329	Shin SI	281	Walker J	159
Marin G	13, 192, 193	Pouyssegur J	266	Shiroki K	208	Wang E	264
Marshall T	184	Pouyssegur JM	236	Shopsis C	228	Wang LH	48, 50, 51, 52
Martin GS36	46, 103, 115, 215, 251	Prasad I	186, 202	Siegler R	88	Warren L	240, 242
Martin MA	323	Purchase HG	128	Singer SJ	265	Wartiovaara J	288
Martin RG19	40, 80, 106, 111, 279, 326	Quigley JP	292	Singh M	294	Watanabe S	16, 196
Mason WS34	97, 98, 99, 310	Rabek J	81, 208	Singh VN	294	Watson KE	96
Matsuya Y	67	Rabinowitz Z	129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 243	Smith HS	90, 218	Watterson DM	276
Mattern CF	157, 158	Rachmeler M	274	Smith RE	276	Weber J	165
May JT	122, 252	Rapp F	31, 32, 322	Smoler D	96	Weber K	107, 261, 262
Mayer AJ	188	Raskas HJ	217	Somers KD63	122, 252, 274	Weber M	251, 254
McMillin C	47	Ray DA	301	Sompayrac L	59	Weber MJ238	249, 256, 257
McNutt NS	151, 260	Reiber M	224	Spear PG	211, 214	Weinstein IB	170
Medina D	12	Reich E	292	Spence C	39	Weintraub H	298
Meganathan R	300	Renger HC	166, 167, 168	Spiegelman S	96	Weiss RA	34, 310, 311
Metroka C	306	Reynolds RK	9, 155	Spira G	63	Weissbach A	61, 189
Mignatti P	284	Rhim JS	164	Spurr N	77	Westmoreland D	32, 322
Miki T	219	Rieber M	291, 291	Stehelin D	53, 54, 55, 181	Wiblin CN	194
Milne BS	127	Rifkin DB	231, 292	Stein S	279	Wickus GG	222, 223
Minekawa Y	94	Riggs JI	23	Steiner SH	282	Wickus GW	304
Minna J	184	Risser R	144, 281	Stephenson JR9	64, 100, 121, 154, 155	Wiener H	295
Miyashita K	174	Robb JA	19, 78, 90	Stoker MG	328	Wilkie NM	60
Moens W	272	Robberson DL	58	Subak-Sharpe JH	60	Williams JH	25, 42, 43
Montagnier L	229	Robbins PW222	223, 248, 304	Suzuki E	24	Willingham MC	266
Mora PT	80, 234	Roberts-Dorv C	218	Suzuki K	196	Wilson JH	177
Morgan HR	245, 246	Robin R	247	Sylvester SS	182	Winkelhake JL	256
Mosser AG	65	Rohrschneider I	290	Takahashi M	28, 91	Wolman S	142
Muller R	61	Rongey RW	10	Takemori N	23	Wray VI	241
Munyon W	209, 210	Rosen P	114	Takemoto KK	71, 83	Wright SE	47
Munyon WH	44	Rosenwirth B	81	Tegtmeyer P68	69, 75, 76, 108	Wyke A	226
Nakajima K	17	Roth SI	297	Temin HM	65, 313	Wyke J	141
Nakajima S	286	Rothschild H	203, 204, 293	Tenen DG	72	Wyke JAS5	221, 290, 310, 311
Nass MM	280	Rowe WP	190	Terry AH	233	Yahara I	263
Nathans D	93, 325	Rubenstein FE	27	Tevethia MJ	79	Yamada KM	237, 266
Neiman PE	47	Rubin H	1, 251	Tevethia SS	79	Yamada SS	237, 266
Nelson-Rees WA	149	Rundell K	75	Timbury MC	213	Yamaguchi N	92, 170, 250
Neuhauer RI	206	Ruoslahti E	288	Tobin A	292	Yamahuchi N	74
Nomaguchi H	11	Sachs I12	129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140	Todaro GJ	83, 148	Yamamoto T	140
Nomura S156	157, 158, 178, 191, 205, 315	Sacks I	243	Tokuno S	17	Yamanishi K	28
Noonan CA	173	Salzberg S	217	Toni R	159	Yaniv A	96
Norkin L	46	Sambrook J	176	Toniolo D	169	Yasue H	94
Okada YS	113	Saral R	40	Torpier G	229	Yau TM	249, 257
Okazaki T	220	Schaffer PA	29, 30, 212	Toxoshima K3	4, 6, 11, 126, 225, 271, 275	Yeoh G	300
Okazaki W	128	Schaffhausen BS	89	Trkulja D	17	Yoshida M11	228, 273, 275
Onodera K	250	Scheele CM	306	Tronick SR	100	Yoshio S	116
Osborn M	107, 261	Scher CD	90, 149	Tsuchida N	161	Yoshike K	196
Ossowski L	292	Scholnick EM	183	Tuttle-Fuller N	178	Young CS	42, 43, 321
Otten J	269			Uehida S	16, 196	Zarling DA	65
Owada M11	126, 225, 273, 275			Uetake H	286	Zouzas D	186, 202, 207
Ozman MN	71			Unkeless JC	292		
Ozanne B	162, 176			Vaheri A	288		
				Van Der Vliet P	81		